MASITINIB SCIENTIFIC DATA FOR VETERINARY MEDICINE

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(for US residence only)

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AB Science is developing its lead compound masitinib simultaneously in veterinary and human medicine. Masitinib is an oral tyrosine kinase inhibitor that targets a limited number of key kinases implicated in various cancers. Its main cellular target is the mast cell, which is capable of releasing large amounts of pro-inflammatory mediators with a consequence of triggering and sustaining an inflammatory response. Owing to its broad mechanisms of action masitinib is developed in various indications, in the fields of oncology and inflammatory disease, in both dogs and cats.

In the spirit of AB Science’s commitment of sharing information with the veterinary community and updating you with most recent developments from our own varied veterinary research program as well as findings communicated from external collaborators and independent practitioners, we have created this information pack of masitinib-related publications and other sources of printed information. It is intended to provide a convenient one-stop reference source, covering peer-reviewed journal articles, meeting abstracts, conference proceeding manuscripts, press articles, and press-releases communicated by international news agencies. With periodic updates we hope to help keep the veterinary community abreast of the latest clinical developments, safety-related issues, and highlight emerging new therapeutic directions of relevance to the veterinary application of masitinib.

Everyone knows that a picture is worth 1000 words, and this saying has never been truer than when comparing before and after images from successful treatment of tumors or inflammatory conditions. However, in an effort to keep this information pack to a manageable size, the presentation of materials rich in photographic evidence and graphical data has been minimized. Many examples of such material, including poster presentations and videos, can be found on the AB Science website (www.ab-science.com), and also on the scientific book ‘The Efficacy of Targeted Therapy in Veterinary Medicine’, which is available on demand (contact@ab-science.com).

From the following summary of publications it can be seen that masitinib holds therapeutic potential for both veterinary oncological and immune-mediated conditions (note that this is a compendium of veterinary communications only; for information on publications relating to human medicine please visit the AB Science website - www.ab-science.com). Emerging clinical evidence, generated largely through the research of veterinary clinical investigators, continues to expand the range of canine or feline diseases for which masitinib may be of therapeutic benefit, either as a single agent or in combination with existing therapies. As such, we believe that masitinib is likely to become an increasingly important tool in the veterinarian’s therapeutic armamentarium.

Masitinib (Masivet® and Kinavet-CA1®) can only be obtained by practitioners of veterinary medicine located in countries for which it has received appropriate authorization. Masitinib is available directly from the AB Science website following client registration. The process is very simple and only requires a few minutes of your time.

Pet owners are advised to contact their local Veterinary Surgeon.

For more information visit www.ab-science.com or contact us at masivet@ab-science.com.
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2 INTRODUCTION

Panels taken from the invited oral presentation and associated poster ‘Masitinib – targeted therapy in veterinary medicine’, that Dr Albert Ahn delivered at the 2011 World Small Animal Veterinary Association World Congress (October 14-17).

Masitinib – Targeted Therapy in Veterinary Medicine

The first ever approved anticancer drug in veterinary medicine - designed specifically to target mast cell dysfunctions

Masitinib is a cutting-edge technology in the treatment of canine mast cell tumor, working directly at the origin of the tumor. Masitinib belongs to the family of drugs called receptor tyrosine kinase inhibitors.

Masitinib is a targeted therapy that limits collateral damage to healthy tissue. This is an important distinction from cytotoxic chemotherapies, which inhibit multiplication of a great many cells including healthy ones.

Masitinib targets just a few key tyrosine kinases that are implicated in various diseases, while not inhibiting, at therapeutic doses, those tyrosine kinases attributed to possible toxicity. This permits masitinib to be administered at higher doses for a more potent therapeutic effect, whilst maintaining an acceptable tolerability and better safety profile compared with multi-targeting drugs or traditional chemotherapies.

Masitinib has been extensively tested to prove its efficiency and safety for use in veterinary medicine. Clinical trials have scientifically proven it to be a valuable anti-cancer treatment with possible long term benefits.

Masitinib significantly improved survival time at 6, 12 and 24 months compared to placebo. At least 50% of dogs treated with masitinib had their tumor half in size during the first 6 months.
Efficacy of masitinib leads to long term tumor control. Responses vary from complete disappearance of the tumor to no impact at all. However, clinical studies demonstrated that 62% of dogs treated by masitinib were still alive after 12 months of therapy.

Owing to its novel mechanism of action, masitinib may be of therapeutic benefit to a number of other cancers (in monotherapy or combination therapy). Masitinib also has potential to treat immune-mediated conditions, as demonstrated in the successful phase 3 study of canine atopic dermatitis.

Numerous phase 1, 2, and 3 studies, supported by preclinical data, help make-up a varied development program investigating masitinib's potential in numerous canine and feline oncology and non-oncological diseases. These include: T cell lymphoma, melanoma, hemangiosarcoma, histiocytic sarcoma, osteosarcoma, bladder cancer, mammary tumors, atopic dermatitis, inflammatory bowel disease, injection-site sarcoma, and asthma.

Masitinib is easy to use and is administered by the client at their home.

Due to the way masitinib works, it is likely that a treatment regimen of at least 6 months will be required for dogs responding to treatment.

Masitinib is generally well tolerated with medically manageable side-effects. The most common side effects that may occur with masitinib include diarrhea and vomiting. To minimize the risk of possible adverse drug effects, and in particular protein loss syndrome, regular checkups are required.

Masitinib can only be obtained by veterinarians. Masitinib is available directly from the AB Science website following client registration. For more information visit www.ab-science.com or contact masivet@ab-science.com or kinavet@ab-science.com (US residence).
Already documented as a valuable anticancer therapeutic for canine mast cell tumor, a wide range of other cancers and immune-mediated disorders are currently being investigated.

- **Development program of masitinib in veterinary medicine** *

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* This list of indications reflects masitinib’s veterinary development program and should not be interpreted.

- **Case studies of masitinib treatment in canine mast cell tumor †**

† MCT case study photographs courtesy of: M. Brearley (Cambridge, UK); J. de Vos, (Terneuzen, Netherlands).
Tyrosine kinase inhibitors (TKI) are an innovative class of drug that has recently become available to veterinary medicine. This targeted therapy inhibits enzymes involved in cellular signaling pathways that regulate key cell functions and survival. Over the last decade TKIs have revolutionized the management of certain human cancers and are now making inroads into veterinary medicine, with canine mast cell tumors (MCT) being their first major success story. Masitinib has been specifically developed for the veterinary setting. Moreover, with its two completed phase III studies in canine oncology and immune-mediated indications, its long-term follow-up of MCT treatment (approximately 6 years), a wealth of knowledge accumulated from over 2 years of on- and off-label use in Europe, and development program for numerous other canine and feline diseases, masitinib serves as a model example of what this remarkably versatile class of therapeutic drug promises to accomplish in veterinary medicine.

Owing to its novel mechanism of action, masitinib holds therapeutic potential for both veterinary oncological and immune-mediated conditions. Emerging clinical evidence also indicates masitinib may be of therapeutic benefit for canine T-cell lymphoma, neurofibrosarcoma, and melanoma; for feline MCT and asthma; and as a chemosensitizer for standard chemotherapies.
3 RECENT NEWS AND UPCOMING EVENTS

3.1 PUBLICATIONS NEW TO THIS ISSUE

- Publication of findings from an *in vitro* study on feline vaccine associated sarcoma in the journal Veterinary and Comparative Oncology (J. Lawrence et al.). Manuscript title: ‘Masitinib demonstrates antiproliferative and proapoptotic activity in primary and metastatic feline vaccine associated sarcoma cells’.


- Presentation (A. Vance) on the potential role of masitinib in the management of feline injection site sarcoma at the 2011 GVSP symposium, August 5-6. Abstract title: ‘In vitro investigation of the use of masitinib and radiation on feline injection site sarcoma cells’.


- Presentation (C. Fahey) at the Veterinary Cancer Society Conference, Albuquerque, 4-7 November 2011. Abstract title: ‘Evaluation of the receptor tyrosine kinase inhibitor, masitinib mesylate, in canine osteosarcoma cell lines’.


3.2 FUTURE PUBLICATIONS AND CONGRESS PRESENTATIONS

- Manuscript by C. Reinero et al., entitled: ‘The tyrosine kinase inhibitor masitinib blunts airway inflammation and improves associated lung mechanics in a feline model of chronic allergic asthma’. In Press journal International Archives of Allergy and Immunology,

- Publication of full research findings from a study investigating the activity of masitinib against canine HSA cell lines (S. Lyles et al.). Manuscript provisionally entitled: ‘In vitro effects of the tyrosine kinase inhibitor, masitinib mesylate, on canine hemangiosarcoma cells’ is currently under journal review.

- Publication of full research findings from two phase 1 studies to determine the maximum tolerated dose of the chemotherapies carboplatin or doxorubicin in combination with masitinib (A. Rusk et al.). Manuscript provisionally entitled: ‘Masitinib in combination with carboplatin or doxorubicin in advanced canine tumours’ is currently under journal review.

- Invited review in Kirk’s Current Veterinary Therapy XV, in the indications & use of masitinib in veterinary medicine (K. Hahn).
Publication of preclinical results showing that masitinib has potential role in the clinical management of feline injection site sarcoma

AB Science SA (NYSE Euronext - FR0010557264 - AB), a pharmaceutical company specializing in the research, development and commercialization of protein kinase inhibitors (PKIs), announces publication in Veterinary and Comparative Oncology of results from a preclinical study showing that masitinib has potential role in the treatment of primary and metastatic feline injection site sarcoma (ISS).

This study, conducted by Dr Jessica Lawrence, (DVM, DACVIM Oncology, DACVR Radiation Oncology; University of Georgia, US) and colleagues, investigated the effects of masitinib on a specific cellular signaling pathway (PDGF/PDGFR), that regulates key cell functions and cell survival, in two feline ISS cell lines. Results showed masitinib was capable of interrupting this signaling pathway resulting in the inhibition of feline ISS proliferation and increased cell death. Masitinib may therefore have a possible role in the management of cats with ISS.

Dr Jessica Lawrence declared: “We have demonstrated that masitinib targets and successfully interrupts an important signaling pathway in the development of feline ISS. Given the availability of masitinib to veterinary professionals, this ability to disrupt PDGFR signaling may represent a potential therapy for cats with ISS in combination with standard radiation therapy and surgery. Identifying the best way to incorporate masitinib into the treatment of cats with ISS is now the focus of new studies with the possibility that this research could lead to the development of treatment with wider applicability in veterinary and human oncology.”


Masitinib was the first ever approved anticancer drug in veterinary medicine, receiving approval from the European Medicines Agency (EMA) under the trade name Masivet®. Masitinib has also recently become obtainable in the United States under the trade name Kinavet CA-1, having received conditional approval in December 2010 from the US Food and Drug Administration (FDA) for treatment of recurrent or nonresectable Grade II and Grade III cutaneous mast cell tumors in dogs that have not previously received radiotherapy and/or chemotherapy except corticosteroids. AB Science is developing masitinib in veterinary medicine in oncology, including several studies to further investigate masitinib’s potential as a chemosensitizer, as well as in non oncology diseases, such as canine atopic dermatitis or asthma in cats.

(Full press release available online or upon request to AB Science - contact@ab-science.com).
AB Science SA (NYSE Euronext - FR0010557264 - AB), a pharmaceutical company specializing in the research, development and commercialization of protein kinase inhibitors (PKIs), announces the publication of a wide-ranging review article entitled ‘Masitinib – a targeted therapy with applications in veterinary oncology and inflammatory diseases,’ in the peer-reviewed journal CAB Reviews and on the veterinary continuing education website VetMed Resources (the most comprehensive online veterinary information service).

Professor Barbara E. Kitchell (DMV, PhD, DACVIM; Michigan State University, USA) declared: «Perhaps the most compelling aspect of masitinib from a veterinarian’s viewpoint is the possible diversity of its applications. Already documented to be a valuable anticancer therapeutic for mast cell tumor treatment of dogs, masitinib also shows real promise as effective treatment of canine atopic dermatitis. Considering also the wide range of other cancer and anti-inflammatory treatments currently being explored by veterinary investigators, masitinib seems likely to become an increasingly important tool in the veterinarian’s therapeutic armamentarium. »

Authored by leading experts in the fields of veterinary oncology, veterinary dermatology, and tyrosine kinase inhibitor research, this article reviews the clinical development of masitinib in veterinary medicine to date, discussing key results from its canine mast cell tumor and atopic dermatitis phase 3 studies, safety-related issues, and emerging new therapeutic directions.

Commenting on the significance of masitinib to the veterinary community, Robert Taylor, editor of CABI’s VetMed Resource, stated that: «We invited this review on masitinib and its use in dogs, because of the current interest within the veterinary profession on this new class of anti-neoplastic agents and the promise they have in treating common cancers such as mast cell tumors. Veterinarians are increasingly interested in reviewing the published literature so that they can base their practice on the best available evidence. »

Professor Olivier Hermine, President of the scientific committee of AB Science commented: «This paper makes extensive use of source material from investigators in the field who are sharing their experiences of masitinib treatment with the scientific community. The importance of such communications cannot be underestimated as they help identify new therapeutic opportunities and we therefore welcome dialogue with investigators planning to initiate studies. Due to the broad therapeutic possibilities of masitinib such interactions may even be essential if we wish to maximize this drug’s benefits in human medicine – experiences and insights from the veterinary setting often serving as a catalyst for further clinical development that can result in tangible benefits for human health. We have already seen an example of such cross-fertilization with the initiation of AB Science’s phase 3 study in human metastatic melanoma. »

This publication is available from the online library of either VetMed Resources or CAB Reviews (www.cabi.org/vetmedresource) (www.cabi.org/cabreviews) and can be cited as: Ogilvie GK et al. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 2011, 6, No. 021, doi: 10.1079/PAVSNNR20110021. A pre-publication author version is also available to download from the following weblink: Click to download
Masitinib decreases signs of canine atopic dermatitis
Publication of phase 3 results in Veterinary Dermatology

AB Science SA (NYSE Euronext - FR0010557264 - AB), a pharmaceutical company specializing in the research, development and commercialization of protein kinase inhibitors (PKIs), announces the publication of results from its phase 3 clinical trial investigating the treatment of canine atopic dermatitis with masitinib in Veterinary Dermatology, the lead journal in this field of research. The article entitled, ‘Masitinib decreases signs of canine atopic dermatitis: a multicentre, randomized, double-blind, placebo-controlled phase 3 trial’, shows that masitinib has potential as an effective treatment of atopic dermatitis. Important facts about this study include:

• Pivotal phase 3 study with one of the largest atopic dermatitis cohorts tested to date, 306 dogs, and a highly robust study design
• Masitinib achieved statistically significant reduction in the signs of canine atopic dermatitis
• Positive response to masitinib was evident for dogs resistant or intolerant to ciclosporin and/or corticosteroids, and dogs with severe pruritus; two populations with high unmet medical need
• Masitinib could provide an important new tool in the veterinarian’s armamentarium for effective treatment of canine atopic dermatitis

The treatment of generalized canine atopic dermatitis remains a challenge, especially in severe or refractory cases. Beyond the already developed therapeutic strategies, there exists an unmet medical need to identify alternative treatments. 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. Masitinib, a selective oral tyrosine kinase inhibitor, effectively inhibits the survival, migration and activity of mast cells.

Dr. Pierre Cadot (Dr.med.vet., Clinique vétérinaire Europa, France) the article’s lead author declared: “The key message from this study has to be that daily administration of oral masitinib achieved significant reduction in the signs of canine atopic dermatitis and therefore could be an effective treatment option. This positive response was evident not only in dogs that had never received treatment before but also in dogs resistant or intolerant to ciclosporin or corticosteroids, and dogs with severe pruritus, two groups representing populations with high unmet medical need”.

Dr. Patrick Hensel (Dr.med.vet., DACVD, University of Georgia, USA) the study’s coordinating investigator commented: “This study was up to date one of the largest randomized, controlled clinical drug trials in the treatment of canine atopic dermatitis. Animals were carefully selected to meet the required criteria of atopic dermatitis. The findings of this study indicate that masitinib, which is selectively targeting mast cells, decreased clinical signs in the study population”.

AB Science announces presentation at the 2011 ACVIM Forum of two invited talks on veterinary use of masitinib

AB Science SA (NYSE Euronext - FR0010557264 - AB), a pharmaceutical company specializing in the research, development and commercialization of protein kinase inhibitors (PKIs), announces that two invited presentations on the topic of veterinary applications of masitinib will be delivered by Dr Albert Ahn at the American College of Veterinary Internal Medicine (ACVIM) Forum to be held in Denver, USA next month (www.acvim.org/websites/forum2011). Full length manuscripts for both of these presentations will be published in the 2011 ACVIM Forum Proceedings.

Dr Albert Ahn (DVM, President of AB Science USA) commented: «Since masitinib received conditional approval for treatment in canine mast cell tumor from the US Food and Drug Administration (FDA) in December 2010, as well as the recent publication of impressive long term survival data in that disease and completion of a successful pivotal trial for treatment of canine atopic dermatitis, there has been a real demand from the veterinary community for more information about this remarkably versatile drug. These invited presentations and their accompanying manuscripts go some way to quench that thirst. »

As part of this initiative on information transfer, AB Science has also created an information pack of masitinib-related veterinary publications titled ‘Masitinib Scientific Data for Veterinary Medicine’, intended to provide a convenient one-stop reference source, covering peer-reviewed journal articles, meeting abstracts, conference proceeding manuscripts, press articles, and press-releases communicated by international news agencies. The next edition is scheduled for release in July and is available upon request from contact@ab-science.com.

The first 2011 ACVIM Forum talk, entitled ‘Masitinib in Canine Mast Cell Disease’, will be part of the Oncology - Specialty Symposium (Wednesday 15th June at 2:45 pm). Masitinib was specifically designed to target mast cells, working therefore directly at the origin of the tumor, in contradistinction to standard cytotoxic chemotherapies that inhibit replication of all cells, including healthy and useful ones. This talk will review data from the successful pivotal trial that evaluated 202 dogs with grade II/III cutaneous mast cell tumor, long term findings at 12 and 24 months from the follow-up study, and case studies exemplifying survival of over 4.5 years in dogs from the pivotal trial’s extension phase. The manuscript accompanying this talk will be published as part of the 2011 ACVIM Forum Proceedings and can also be downloaded from the following link: 2011 ACVIM Forum - Masitinib in Canine Mast Cell Disease

The second 2011 ACVIM Forum talk is part of the Technician Program, in which Dr Albert Ahn presents a comprehensive review on the emerging role of tyrosine kinase inhibitors in veterinary medicine and in particular masitinib. Topics will range from findings of the two completed phase III studies in canine oncology and immune-mediate indications, as well as the development program for numerous other canine and feline oncology and non-oncological diseases. The talk is entitled ‘New Therapeutic Options in Veterinary Oncology: Tyrosine Kinase Inhibitors’, and is scheduled for Saturday 18th June at 5:25 pm. The manuscript accompanying this talk will be published as part of the 2011 ACVIM Forum Proceedings and can also be downloaded from the following link: 2011 ACVIM Forum - Masitinib Scientific Data for Veterinary Medicine
Publication of preclinical results demonstrates that masitinib can act as a potentiator of chemotherapies

AB Science SA (NYSE Euronext - FR0010557264 - AB), a pharmaceutical company specializing in the research, development and commercialization of protein kinase inhibitors (PKIs), announces publication in The Veterinary Journal of results from a preclinical study showing that masitinib has potential as a chemosensitizer.

This study, conducted by Dr Douglas Thamm (VMD, Diplomate ACVIM Oncology; Colorado State University) and colleagues, investigated the ability of masitinib to sensitize different canine cancer cell lines to various chemotherapeutic agents. Results showed that masitinib sensitized numerous tumor cell lines from different origins (breast, bladder, melanoma, lymphoma, etc.) to chemotherapeutic drugs such as doxorubicin, gemcitabine and vinblastine. These data also provide additional weight to findings from studies showing that masitinib can enhance the antiproliferative effects of gemcitabine in human pancreatic cancer, including gemcitabine-resistant cell lines, which is a property not seen with other tyrosine kinase inhibitors (Humbert et al. PLoS One, 2010).

Dr Douglas Thamm declared: « This study provides further evidence that masitinib can exert an anticancer action that extends beyond the inhibition of its main tyrosine kinase targets by acting in synergy with standard chemotherapies. Such chemosensitization may allow lower concentrations of chemotherapeutic agent to be used, thereby reducing toxicity risks, or may increase the available efficacy at standard doses ». 

Professor Olivier Hermine, President of the scientific committee of AB Science commented: « These data taken together with findings in human pancreatic cancer cell lines are highly significant because one of the main limitations to certain chemotherapy treatments is drug resistance, and so a drug capable of counteracting resistance would facilitate the prolonged therapeutic benefits of such chemotherapies ».


To read more about publications of masitinib in human and veterinary medicine visit www.ab-science.com

(Full press release available online or upon request to AB Science - contact@ab-science.com).
Publication of Long-Term Survival of Dogs with Mast Cell Tumours Treated with Masitinib

AB Science SA (NYSE Euronext - FR0010557264 - AB), a pharmaceutical company specialising in the research, development and commercialisation of protein kinase inhibitors (PKIs), announces publication of increased long-term survival with masitinib in canine mast cell tumour.

In new research published in the peer-reviewed scientific journal American Journal of Veterinary Research, Dr Kevin Hahn (DVM, PhD) and colleagues report on the effectiveness of masitinib for the treatment of nonresectable mast cell tumours (MCTs) in dogs at 12 and 24 months after onset of treatment. This was a follow-up study from the successful pivotal 6-month, phase III, placebo-controlled clinical trial of masitinib in the treatment of dogs with nonmetastatic grade 2 or grade 3 cutaneous MCTs. Masitinib, arguably the most specific inhibitor of c-Kit available, was subsequently registered by the European Medicine Agency (EMA) under the trade name Masivet®, making it the first approved targeted therapy in veterinary oncology. Masitinib recently received conditional approval from the Food and Drug Administration (FDA) for commercialisation under the trade name Kinavet-CA1.

Two major conclusions are drawn from this publication. First, the long-term follow-up data provides further evidence that masitinib is effective in the treatment of nonresectable MCTs and provided benefits in terms of long-term survival. Moreover, masitinib treatment was shown to improve long-term survival regardless of the subtype of MCT being treated (with respect to c-Kit mutational status). Second, analysis revealed that control of disease at 6 months was highly predictive of long-term survival, whereas short-term response at 6 weeks was not.

Alain Moussy, Chairman and CEO of AB Science declared: « In the field of oncology this survival is critically important to establish a drug as a benchmark. Recent examples have shown that oncology products could reduce tumour size in the short-term at a cost of toxicity but could not increase survival over the longterm or even worse reduce survival. It is relatively difficult to generate these long-term survival data because you need to follow patients in a comparative manner (that is, masitinib versus existing standard of care) for years, which AB Science has done. Significant improved survival has been demonstrated, which is good news for dogs suffering from mast cell tumours ».

Publication is available from the AJVR website:

(Full press release available online or upon request to AB Science - contact@ab-science.com).
Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)


BACKGROUND: The stem cell factor receptor, KIT, is a target for the treatment of cancer, mastocytosis, and inflammatory diseases. Here, we characterise the in vitro and in vivo profiles of masitinib (AB1010), a novel phenylaminothiazole-type tyrosine kinase inhibitor that targets KIT.

METHODOLOGY/PRINCIPAL FINDINGS: In vitro, masitinib had greater activity and selectivity against KIT than imatinib, inhibiting recombinant human wild-type KIT with an half inhibitory concentration (IC(50)) of 200±40 nM and blocking stem cell factor-induced proliferation and KIT tyrosine phosphorylation with an IC(50) of 150±80 nM in Ba/F3 cells expressing human or mouse wild-type KIT. Masitinib also potently inhibited recombinant PDGFR and the intracellular kinase Lyn, and to a lesser extent, fibroblast growth factor receptor 3. In contrast, masitinib demonstrated weak inhibition of ABL and c-Fms and was inactive against a variety of other tyrosine and serine/threonine kinases. This highly selective nature of masitinib suggests that it will exhibit a better safety profile than other tyrosine kinase inhibitors; indeed, masitinib-induced cardiotoxicity or genotoxicity has not been observed in animal studies. Molecular modelling and kinetic analysis suggest a different mode of binding than imatinib, and masitinib more strongly inhibited degranulation, cytokine production, and bone marrow mast cell migration than imatinib. Furthermore, masitinib potently inhibited human and murine KIT with activating mutations in the juxtamembrane domain. In vivo, masitinib blocked tumour growth in mice with subcutaneous grafts of Ba/F3 cells expressing a juxtamembrane KIT mutant.

CONCLUSIONS: Masitinib is a potent and selective tyrosine kinase inhibitor targeting KIT that is active, orally bioavailable in vivo, and has low toxicity.
4.2 MASITINIB AS A CHEMOSENSITIZER OF CANINE TUMOR CELL LINES

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)


SUMMARY
Masitinib, a selective tyrosine kinase inhibitor, has previously been shown to enhance the antiproliferative effects of gemcitabine in human pancreatic cancer, demonstrating potential as a chemosensitizer. This exploratory study investigated the ability of masitinib to sensitize various canine cancer cell lines to doxorubicin, vinblastine, and gemcitabine. Masitinib strongly sensitized histiocytic sarcoma cells to vinblastine (>70-fold reduction in IC₅₀ at 5 μM masitinib), as well as osteosarcoma and mammary carcinoma cells to gemcitabine (>70-fold reduction at 5–10 μM). In addition, several cell lines were sensitized to doxorubicin (2–10-fold reduction at ≤10 μM). These data establish proof-of-concept that masitinib in combination with chemotherapeutic agents can generate synergistic growth inhibition in various canine cancers, possibly through chemosensitization. The findings justify further investigation into those combinations that may potentially yield therapeutic benefit.

4.3 GAIN-OF-FUNCTION MUTATIONS IN THE EXTRACELLULAR DOMAIN OF KIT ARE COMMON IN CANINE MAST CELL TUMORS

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)


SUMMARY:
In the current study, we examined the types and frequency of KIT mutations in mast cell tumors from 191 dogs. Sequencing of reverse transcription-PCR products revealed alterations in 50 (26.2%) of the dogs. Most mutations were in exon 11 (n = 32), and of these, most were internal tandem duplications (n = 25) between residues 571 and 590. Within exon 11, there were two hotspots for mutations at codons 555-559 and 571-590. In addition, nine dogs had mutations in exon 8 and eight had mutations in exon 9. We selected the two most common mutants and two representative exon 11 mutants for further analysis. When expressed in Ba/F3 cells, they were constitutively tyrosine phosphorylated and induced growth factor-independent cell proliferation. AG1296, a tyrosine kinase inhibitor, dose dependently inhibited both the tyrosine phosphorylation of these mutants and their induction of growth factor-independent proliferation. This study shows that activating mutations in not only exon 11 but also exons 8 and 9 are common in canine mast cell tumors. These results also show that Ba/F3 cells can be used for the direct characterization of canine KIT mutants, eliminating the need to make equivalent mutations in the mouse or human genes.
4.4 A TYROSINE KINASE INHIBITOR TARGETING C-KIT FOR CHRONIC INFLAMMATORY DISEASES INVOLVING MAST CELLS

Publication Category: Conference Presentation

Proceedings of the 24th North American Veterinary Dermatology Forum, 2009
Olivier Hermine

SUMMARY

Abstract: Mast cell proliferation, differentiation, and degranulation (the release of inflammatory cytokines and mediators that may have a physiological or pathological effect) is regulated by the growth factor receptor, c-Kit. Inhibitors of the c-Kit receptor may therefore have a beneficial effect in diseases conditions that involve mast cells. The objective of this work was to demonstrate the biological benefits of masitinib administration in three different in vivo rodent models of chronic inflammatory diseases.

In one model of allergic airway inflammation, Balbc mice sensitized to ovalbumine were given masitinib (25 or 100 mg/kg/day, p.o., after the sensitization process). Masitinib diminished significantly the airway hyperresponsiveness (30% reduction) during a methacholine challenge test (measured by the bronchconstrictive response) and significantly reduced the number of eosinophils the bronchoalveola lavage fluid (70% reduction) (n.5, p<0.05).

Similarly, in another model of dextran sodium sulphate-induced colitis in Balbc mice, masitinib dose dependently (25mg/kg and 50m/kg) significantly reduced the symptoms (10% and 50% reduction of weight loss) and extent of macroscopic and histological lesions (25% and 50%) as well as colonic concentrations of myeloperoxidase (MPO) and inflammatory cytokines including TNF alpha (n.10; p<0.05).

The results obtained from these in vivo studies in addition to our previous report regarding the beneficial symptomatic improvements noted in dogs with atopic dermatitis provide a rationale to perform studies with of c-kit inhibitors in other chronic inflammatory disorders involving mast cells in dogs including asthma and inflammatory bowel diseases.
**4.5 MASITINIB IS A CHEMOSENSITIZER OF CANINE TUMOR CELL LINES**

Publication Category: Conference Presentation

Proceedings of the 28th Annual Conference of the Veterinary Cancer Society, 2008
Douglas H Thamm, Barbara Rose, Kelvin Y Kow, Martine Humbert, Alain Moussy, Olivier Hermine, Patrice Dubreil

**INTRODUCTION**: We recently showed that masitinib, a tyrosine kinase inhibitor targeting c-Kit, PDGF receptor and FGFR3 and affecting the FAK pathway, is safe and effective for the treatment of grade II or III nonresectable or recurrent mast cell tumors in dogs. To investigate the possible use of masitinib in combination with chemotherapy in dogs, we examined its ability to sensitize canine cancer cell lines to doxorubicin, vinblastine, and gemcitabine.

**METHODS**: A variety of canine tumor cells were incubated for 72 h with varying concentrations of chemotherapeutic agent +/- masitinib. Relative viable cell number was then determined using a commercial bioreductive fluorometric assay, expressed as a percentage versus untreated cells, and 50% inhibitory concentrations calculated using nonlinear regression, fitting the data to a sigmoidal dose-response curve.

**RESULTS**: Masitinib demonstrated potent single-agent antiproliferative activity against OSW canine T-cell lymphoma. Masitinib (1-10 μM) sensitized all evaluated cell lines to doxorubicin (2- to 10-fold reduction in IC50), strongly sensitized DH82 histiocytic sarcoma cells to vinblastine (>74-fold reduction), and strongly sensitized D17 and Abrams osteosarcoma and CMT12 and CMT27 mammary carcinoma cells to gemcitabine (>10-fold reduction).

**CONCLUSIONS**: Masitinib can chemosensitize canine tumor cell lines to the antiproliferative effects of multiple antineoplastic drugs at clinically achievable concentrations. The OSW canine T cell lymphoma cell line demonstrates unique sensitivity to masitinib through an s-yet unknown mechanism. The encouraging data presented here strongly justify clinical evaluation of masitinib/chemotherapy combinations in dogs with spontaneous tumors.
5 CANINE MAST CELL TUMOR

5.1 MASITINIB IN CANINE MAST CELL DISEASE

Summary
Since their introduction about 10 years ago, tyrosine kinase inhibitors (TKI) have revolutionized the treatment of certain diseases in human medicine and represent a cutting-edge technology in cancer therapeutics. Today, armed with positive findings from two phase III randomized controlled trials, including approximately 6 years long-term follow-up data in canine mast cell tumor, 2 years of European post-market experience, and recently acquired regulatory approval from the US Food and Drug Administration (FDA), the TKI known as masitinib is set to do likewise for veterinary medicine. Drugs of this class inhibit protein tyrosine kinases, enzymes involved in cellular signaling pathways that regulate key cell functions such as proliferation, differentiation, migration, activation and survival. Activation of these enzymes, through mechanisms such as point mutations or over expression, can lead to various forms of cancer as well as non-oncological disorders. The first oncological disorder targeted by this drug has been canine mast cell tumors (MCT). This cancer is the most common cutaneous malignant neoplasm in dogs. However, masitinib has therapeutic potential beyond the field of chemotherapeutics, its highly targeted mechanism of action generating treatment possibilities in a large number of immune-mediated and neurological conditions. This diversity is exemplified via a successful phase III study that showed significant reduction in the symptoms of canine atopic dermatitis following treatment with masitinib.
5.2 EVALUATION OF 12- AND 24-MONTH SURVIVAL RATES AFTER TREATMENT WITH MASITINIB IN DOGS WITH NONRESECTABLE MAST CELL TUMORS

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)


Erratum in:

OBJECTIVE: To evaluate the effectiveness of masitinib for the treatment of nonresectable mast cell tumors (MCTs) in dogs at 12 and 24 months after onset of treatment.

ANIMALS: 132 dogs with nonresectable grade 2 or 3 MCTs.

PROCEDURES: Dogs received masitinib (12.5 mg/kg/d, PO; n = 106) or a placebo (26). After 6 months, treatment was extended with tumor assessments at 3-month intervals until detection of disease progression. Endpoints were tumor response and overall survival rate and time.

RESULTS: In dogs with nonresectable MCTs, masitinib significantly improved survival rate, compared with results for the placebo, with 59 of 95 (62.1%) and 9 of 25 (36.0%) dogs alive at 12 months and 33 of 83 (39.8%) and 3 of 20 (15.0%) dogs alive at 24 months, respectively. Median overall survival time was 617 and 322 days, respectively. Tumor control at 6 months had a high predictive value for 24-month survival, with high specificity (88%) and sensitivity (76%), whereas short-term tumor response (within 6 weeks) had a poor predictive value. Complete responses at 24 months were observed in 6 of 67 (9.0%) dogs with nonresectable MCTs treated with masitinib.

CONCLUSIONS AND CLINICAL RELEVANCE: Masitinib significantly increased survival rates at 12 and 24 months in dogs with nonresectable MCTs. Control of disease at 6 months, but not best response at 6 weeks, was predictive of long-term survival in dogs treated with masitinib, which suggested that short-term response may be irrelevant for assessing clinical efficacy of tyrosine kinase inhibitors for treatment of MCTs.
5.3 MASITINIB IS SAFE AND EFFECTIVE FOR THE TREATMENT OF CANINE MAST CELL TUMORS

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)


Erratum in:

Comment in:

BACKGROUND: Activation of the KIT receptor tyrosine kinase is associated with the development of canine mast cell tumors (MCT).

HYPOTHESIS/OBJECTIVE: To evaluate the efficacy of masitinib, a potent and selective inhibitor of KIT, in the treatment of canine MCT.

ANIMALS: Two hundred and two client-owned dogs with nonmetastatic recurrent or nonresectable grade II or III MCT.

METHODS: Double-blind, randomized, placebo-controlled phase III clinical trial. Dogs were administered masitinib (12.5 mg/kg/d PO) or a placebo. Time-to-tumor progression (TTP), overall survival, objective response at 6 months, and toxicity were assessed.

RESULTS: Masitinib increased overall TTP compared with placebo from 75 to 118 days (P = 0.038). This effect was more pronounced when masitinib was used as first-line therapy, with an increase in the median TTP from 75 to 253 days (P = 0.001) and regardless of whether the tumors expressed mutant (83 versus not reached [P = 0.009]) or wild-type KIT (66 versus 253 [P = 0.008]). Masitinib was generally well tolerated, with mild (grade I) or moderate (grade II) diarrhea or vomiting as the most common adverse events.

CONCLUSIONS AND CLINICAL IMPORTANCE: Masitinib is safe and effective at delaying tumor progression in dogs presenting with recurrent or nonresectable grade II or III nonmetastatic MCT.
5.4 FIFTY MONTHS AND COUNTING: CASE STUDIES EXEMPLIFYING THE LONG-TERM SURVIVAL OF MASITINIB IN DOGS WITH NON-RESECTABLE GRADE II MAST CELL TUMORS

Publication Category: Conference Presentation

Proceedings of the 30th Annual Conference of the Veterinary Cancer Society, October 29-November 1, 2010 (also in Veterinary and Comparative Oncology, 9, 1, e1–e49. DOI: 10.1111/j.1476-5829.2010.00252.x)

Phillips B, Legendre A, Shaw N, Ahn A

INTRODUCTION: In general, the objective of anti-cancer treatment is tumor control leading to prolonged survival and ideally a complete response.

METHODS: Presented are case studies that exemplify the long-term survival, even curative, benefits of masitinib, a tyrosine kinase inhibitor that potently and selectively targets c-Kit and consequently mast cells, in dogs with non-resectable grade II mast cell tumors (MCT).

RESULTS: Each dog has received oral masitinib in monotherapy for over 4 years as part of a pivotal trial’s extension phase. Key results from that study included a decrease in tumor size by ≥50% during the first 6 months in 50% of masitinib-treated dogs, versus 29% in the placebo group (p=0.02). Moreover, follow-up data at 24 months reported six masitinib-treated dogs (9%) maintained complete tumor response compared to none with placebo. Sustainability of this long-term response is further evidenced from the three dogs that remain in the extension phase. The first and second cases presented as non-resectable grade II MCT with c-Kit mutation (exon 11 and exon 8, respectively). Complete response was achieved with masitinib as first-line treatment, which has persisted for approximately 57 months and 52 months, respectively. The third case presented with non-resectable grade II MCT and no c-Kit mutation (wild-type). Masitinib administered as a third-line treatment induced a complete response, lasting now for approximately 53 months. Masitinib has been well tolerated over this time, as evident by the clinical report files.

CONCLUSION: Masitinib significantly improves long-term survival of dogs with non-resectable tumors, irrespective of the presence of c-Kit mutations.
5.5 FIRST-LINE AND RESCUE THERAPY WITH MASITINIB INTEGRATED PROTOCOLS FOR CANINE CUTANEOUS MAST CELL TUMORS.

Publication Category: Conference Presentation

Proceedings of the 30th Annual Conference of the Veterinary Cancer Society, October 29-November 1, 2010 (also in Veterinary and Comparative Oncology, 9, 1, e1–e49. DOI: 10.1111/j.1476-5829.2010.00252.x)
Johan de Vos and Malcolm Brearley

INTRODUCTION: How tyrosine kinase inhibitors will be integrated into standard chemotherapy protocols for canine mast cell tumors (MCT) has yet to be determined. This study investigates masitinib incorporation in first-line and rescue therapy of canine cutaneous MCTs.

METHODS: 44 dogs with incompletely removed or non-resectable cutaneous MCT received masitinib in a variety of settings. Group A: masitinib only (n=11: 2 gr-II, 4 gr-III, 5 gr-unknown). Group B: masitinib/prednisone (n=13: 1 gr-I c-KIT mutated, 3 gr-II, 5 gr-III, 4 gr-unknown). Group C: masitinib/prednisone, plus vinblastine and/or lomustine (n=11: 6 gr-II, 5 gr-III). Group D chemo-resistant masitinib rescue (n=9: 1 gr-II, 5 gr-III, 3 gr-unknown). Survival analyses were performed by Kaplan Meier Product limit method.

RESULTS: 27 dogs achieved CR, 6 PR, 5 SD, and 6 PD. c-KIT status was known in 9 MCTs: 7 mutated all achieved CR, 2 wild-type had SD. There were 17 tumor-related and 2 treatment-related deaths, 5 died from unrelated causes. Twenty dogs remain alive (51-539 days; 4 off treatment). The MSTs for the treatment groups were: A-136 days (4 CR; 3/11 alive); B-not reached (10 CR; 10/13 alive); C-273 days (8 CR; 5/11 alive); D-87 days (5 CR; 2/9 alive for 281 and 334 days). The MST for cases achieving CR was significantly longer than those that did not (NR vs. 81 days; p=0.0001), irrespective of grade or group.

CONCLUSIONS: Completeness of remission, not grade or protocol, was the most significant prognostic factor for MST following masitinib integrated protocols for canine naïve and drug-resistant MCTs.
5.6 REVIEW OF U.S. CASES TREATED WITH MASIVET

Publication Category: Conference Presentation

Proceedings of the 31st Annual Conference of the Veterinary Cancer Society, 4-7 November, 2011.
Barbara Kitchell

INTRODUCTION: Prior to the approval of Kinavet-CA1 by the FDA CVM, masitinib, a phenylaminothiozole-

- type of tyrosine kinase inhibitor was made available to U.S. veterinarians from July 1, 2009 through December
16, 2010 through regulatory discretion. During this time, Masivet® (European approved product) was shipped to
U.S. veterinarians who requested masitinib. Each shipment provided the requesting clinician with an up to 90 day
supply of drug.

METHODS: U.S. veterinarians, predominantly ACVIM-boarded oncologists, contacted AB Science and
provided details of individual patients for whom they were seeking masitinib through regulatory discretion.
Masivet® was then shipped from France to the U.S. and delivered following FDA inspection and customs
clearance. At the conclusion of the program, a survey instrument was sent to all veterinarians who received
masitinib and the data were then collected and analyzed.

RESULTS: During the time period when masitinib was provided under regulatory discretion, 203 institutions
requested the drug for 545 patients. Based on completed surveys for the MCT patients, there were 19% complete
responses; 29% partial responses; 23% stable disease and 29% progressive disease cases. 60% of cases were
treated for non-MCTs, the most common being lymphosarcoma, melanoma, hemangiosarcoma and AGASACA.

CONCLUSIONS: The results of the regulatory discretion program for Masivet® (masitinib) demonstrated that
patients who needed this drug, which was concurrently undergoing regulatory review for the U.S. market, were
able to receive the clinical benefits from this medication. Clinical responses noted in non-MCTs also indicate a
need to further investigate this drug in other canine neoplasms.
5.7 IMPACT OF MASITINIB ON METASTATIC MAST CELL TUMORS AND ON THE EMERGENCE OF METASTASIS FROM NON-METASTATIC TUMORS

Publication Category: Conference Presentation

Proceedings of the 29th Annual Conference of the Veterinary Cancer Society, October 16-19, 2009
Olivier Hermine

INTRODUCTION: Masitinib is a tyrosine kinase inhibitor targeting c-Kit, the growth factor receptor of mast cells (MCs). Masitinib targets both mutated forms of c-Kit found in tumoral MCs and wild type c-Kit in normal MCs. Normal MCs may facilitate the invasion of tumoral cells in surrounding tissues via the release of proteases loosening tissues and angiogenic factors favoring tumor neo-vascularization, one of the steps leading to metastasis. Therefore, masitinib not only targets tumoral MCs, but may also exhibit antimetastatic properties by inhibiting normal MCs. This hypothesis is supported by several observations from clinical trials with masitinib.

METHODS: During the pivotal clinical field study, masitinib (12.5 mg/kg/day) significantly reduced the emergence of nodal/visceral metastases in dogs with recurrent or nonresectable grade 2/3 cutaneous MCT without lymph node or visceral metastases. Only 3.7% of dogs receiving masitinib developed nodal or visceral metastases (versus 17.1% of dogs under placebo; p<0.001).

RESULTS: During a pilot study, masitinib (9.2-12.5 mg/kg/day) induced long-lasting complete remission in two out of three dogs presenting with nodal and/or visceral metastases, including one dog in first-line of treatment and one dog that had received cytotoxic chemotherapy. The dog that showed progression had prior surgical resection and several cycles of cytotoxic chemotherapy. Accordingly, post-marketing experience reported that masitinib (10-12.5 mg/kg/day) induced complete responses in three out of five dogs presenting with metastasized and chemotherapy-resistant MCTs.

CONCLUSIONS: Overall, these data suggest that masitinib exhibits anti-metastatic properties by preventing the emergence of nodal/visceral metastases in non-metastatic tumor and inducing complete remissions of metastatic MCTs.
Publication Category: Conference Presentation

Proceedings of the 29th Annual Conference of the Veterinary Cancer Society, October 16-19, 2009
Olivier Hermine

INTRODUCTION: Masitinib is a tyrosine kinase inhibitor targeting c-Kit, the growth factor receptor of mast cells. Masitinib inhibits both constitutively active mutated forms of c-Kit (found in 25% of mast cell tumors, MCTs) and wild type (WT) c-Kit.

METHODS: The efficacy of masitinib in MCTs was tested in a multicenter, randomized, placebo-controlled, double-blind study. Two-hundred-and-two dogs with recurrent or non-resectable grade 2/3 MCTs, including 139 dogs with WT c-Kit tumors, received either masitinib (12.5 mg/kg/day) or placebo. Endpoints were tumor response/progression (WHO criteria) and survival.

RESULTS: Masitinib administration resulted in maximal blood concentrations (Cmax: 2μM) above the IC50 for WT c-Kit (100 nM). In dogs with WT c-Kit non-resectable tumors, masitinib significantly improved time-to-progression (TTP) with a median of 140 days (versus 75 for placebo, hazard ratio=1.90, p=0.027). At 12 months, 24.0% of dogs had controlled disease, including 10.0% in complete remission. Moreover, 6.5% of dogs remained in complete remission at 24 months. Masitinib improved survival with a median of 779 days (versus 361 for placebo, hazard ratio: 1.26) and survival rates of 61.3% and 42.6% at 12 and 24 months, respectively (versus 43.8% and 16.7% for placebo, respectively). The efficacy of masitinib was enhanced in first-line therapy with further improved TTP (median: 253 versus 66 days for placebo, hazard ratio=2.73, p=0.008) and tripled survival time (median: 937 versus 286 days for placebo, hazard ratio: 1.73).

CONCLUSIONS: In conclusion, masitinib seems effective in the treatment of non-resectable MCTs expressing WT c-Kit, with enhanced efficacy in first-line therapy.
INTRODUCTION: In oncology, clinical studies often use short-term tumor responses as surrogate endpoints for survival. A two-year follow-up from a pivotal filed study evaluating masitinib, a tyrosine kinase inhibitor (TKI) targeting c-Kit, for canine mast cell tumors (MCT) allowed analyzing the predictivity of short-term tumor responses for survival.

METHODS: Two-hundred-and-two dogs having recurrent or non-resectable grade 2/3 cutaneous MCT without nodal/visceral metastases received oral masitinib (12.5 mg/kg/day) or placebo for 6 months and could extend treatment with tri-monthly visits. Endpoints were tumor response/progression (WHO criteria) and survival.

RESULTS: In dogs with non-resectable MCT, masitinib significantly improved survival rates at 12 (62.1% versus 36.0% for placebo, p=0.024) and 24 months (39.8% versus 15.0% for placebo, p=0.040) and almost doubled survival time with a median of 617 days (versus 322 days for placebo). Moreover, 9% of dogs remained in complete remission at 24 months, suggesting these dogs might be cured. Tumor response at 6 weeks was not predictive of long-term survival (Yule: 0.02, p=0.932). Conversely, tumor control at 6 months was highly predictive (Yule: 0.90, p<0.001 for 24-month survival) showing high specificity (90%) and sensitivity (66%). Tumor response and tumor stabilization at 6 months translated into similar survival confirming that sustainable disease stabilization in MCT was predictive of a good prognosis.

CONCLUSIONS: In conclusion, masitinib significantly improved long-term survival in dogs with nonresectable MCT. Controlled disease at 6 months, but not tumor response at 6 weeks, was predictive of long-term survival, suggesting short-term response may be irrelevant for assessing clinical efficacy of TKIs in MCT.
5.10 RESPONSE TO TREATMENT WITH MASITINIB OF CHEMOTHERAPY RESISTANT, METASTASIZED, CANINE CUTANEOUS GRADE 2 AND 3 MAST CELL TUMORS: A PILOT STUDY.

Publication Category: Conference Presentation

Proceedings of the 29th Annual Conference of the Veterinary Cancer Society, October 16-19, 2009
Johan de Vos, Patrick Dubreuil, Alain Moussy, Tom Chapuis, Annette Burm, Lotte van Kuijk, Olivier Hemine

INTRODUCTION: Thirty percent of canine mast cell tumors (MCT’s) display mutations in exon 8, 9, 11 or 17 of c-Kit proto-oncogene. Masitinib is a tyrosine kinase inhibitor, with confirmed efficacy as first-line treatment of canine MCT’s with mutated as well as wildtype c-Kit receptors. Inclusion criteria for this study were dogs with chemotherapy resistant MCT’s.

METHODS: Five dogs with chemotherapy resistant, metastasized, grade 2 and 3 cutaneous MCT’s were treated with masitinib (10-12.5 mg/kg/day). Prior to this treatment, these dogs received prednisolon, vinblastin, lomustin and/or radiation therapy. All MCT’s developed resistance to these treatment modalities. Quality of life of all dogs, previous to masitinib treatment, in fact warranted euthanasia. C-Kit proto-oncogene was sequenced through RTPCR in all five dogs.

RESULTS: In one dog a deletion mutation (DM) and in two dogs an internal tandem duplication (ITD) in exon 11 of c-Kit was detected. The other dogs had wild-type c-Kit. Masitinib induced CR in the dogs with mutated c-Kit. Both dogs with wild-type c-Kit were euthanized shortly after start of masitinib treatment because of MCT progression. In the dogs with ITD, MCT’s recurred after 63 and 92 days, with OST of 79 and 114 days respectively. Post-mortem sequencing was performed in one dog with ITD and revealed an unchanged mutation status. The dog with DM showed no recurrence so far.

CONCLUSIONS: Masitinib appears efficient to induce remission in chemotherapy resistant metastasized canine cutaneous grade 2 and 3 MCT’s with mutated c-Kit.
5.11 EVALUATION OF MASITINIB IN THE TREATMENT OF CANINE MAST CELL TUMORS: LONG-TERM FOLLOW-UP EFFICACY DATA FROM PHASE 3 CLINICAL STUDY

Publication Category: Conference Presentation

Proceedings of the Annual Congress of the European Society of Veterinary Oncology, March 26-29, 2009
O Hermine, JP Kinet, P Dubreuil, A Moussy, KA Hahn.

PURPOSE: We evaluated masitinib for the treatment of canine grade 2/3 mast cell tumors (MCT) in multicenter, randomized, placebo-controlled, double-blind study. Previously reported data at 6-month showed that masitinib significantly improved time-to-progression (Log-Rank p=0.033). Here we report a follow-up at 24-months.

DESIGN: Two-hundred-and-two dogs having recurrent or non-resectable grade 2/3 cutaneous MCT without lymph node or visceral metastases received either masitinib (12.5 mg/kg/day, per os, 161 dogs) or placebo (41 dogs). Endpoints were tumor response/progression (WHO criteria) and overall survival (OS).

RESULTS: Two-year follow-up data confirmed the improvement of time-to-progressing and showed that masitinib induces long-lasting tumor control. Masitinib was especially efficient on non-resectable tumors. At 12-month, 31.6% of dogs under masitinib had controlled disease (versus no dogs under placebo, p<0.001) and 15.8% were in complete remission. At 24-months, 11.8% of dogs remained in complete remission. Masitinib also improved survival. It increased survival rate at 12-months (61.3% versus 37.5% under placebo, p=0.041) and at 24-months (36.4% versus 15.0% under placebo) and almost doubled the survival time with a median survival of 617 days (versus 322 days under placebo). Masitinib was also particularly efficient on tumors expressing a mutated c-Kit. At 12-month, 31.8% of dogs under masitinib had tumor response (versus no dogs under placebo) and 27.3% were in complete remission. Moreover, at 24-months, 23.8% of dogs remained in complete remission. Masitinib also improved survival. It increased survival rate at 12-months (62.9% versus 1.1% under placebo, p=0.008) and at 24-months (29.3% versus no dogs under placebo) and almost tripled the survival time with a median survival of 498 days (versus 182 days under placebo, hazard ratio: 2.91, p=0.009).

CONCLUSIONS AND CLINICAL IMPORTANCE: Masitinib induced a long-lasting control of tumor progression and was curative (complete remission at 24-months) in a subset of patients. When masitinib was used on nonresectable tumors and on tumors expressing a mutated c-Kit, it was particularly efficient and significantly improved long-term survival.
5.12 ASSESSMENT OF RESPONSE TO THE TREATMENT WITH MASITINIB (MASIVET) OF CHEMOTHERAPY RESISTANT, GRADE 2 AND 3, METASTASIZED CANINE CUTANEOUS MAST CELL TUMORS. REPORT OF FOUR CASES

Publication Category: Conference Presentation

Proceedings of the Annual Congress of the European Society of Veterinary Oncology, March 26-29, 2009
J.P. de Vos, A. Moussy, A. Burm, L. van Kuijk, O. Hermine

INTRODUCTION: c-Kit receptor with the ligand stem cell factor is involved in cell division, differentiation and survival of canine mast cell tumors (MCT). Thirty percent of canine MCT display a mutation in exon 8, 9, 11 or 17 of the c-Kit proto-oncogene. masitinib is a novel tyrosine kinase inhibitor, targeting both c-Kit receptor (mutated and wild type) and PDGFRβ, FGFR3 and FAK pathway.

MATERIAL AND METHODS: Four dogs with chemotherapy resistant, metastasized, grade 2 and 3 cutaneous MCT were treated with masitinib (10-12.5 mg/kg/day p.o.). Prior to the treatment with masitinib, these dogs received several cycles of prednisolone, vinblastin, lomustin and radiation therapy. All MCT became resistant to these treatment modalities. In fact, previous to treatment with masitinib the quality of life of all dogs warranted euthanasia. The mutation status of c-Kit has been analyzed in three of the four dogs.

RESULTS: In one dog a deletion mutation in c-Kit (exon 11) was detected; in the other two no mutation was present. masitinib induced CR in two dogs (one had the c-Kit mutation), with a notable response within seven days after start of therapy. PR and SD were obtained in the dogs with wild-type c-Kit. Treatment duration so far is 45 days. Overall tolerability of masitinib was good, and no side effects were noticed in this small study.

CONCLUSION: masitinib seems an efficient drug to induce remission for chemotherapy resistant grade 2 and 3 MCT. A longer follow up is necessary to evaluate whether the remissions are durable.
5.13 MASITINIB IS EFFECTIVE IN THE TREATMENT CANINE GRADE 2/3 MAST CELL TUMOURS THAT ONLY EXPRESS WT C-KIT

Publication Category: Conference Presentation

Proceedings of the British Small Animal Veterinary Association Congress, 2009
O Hermine, JP Kinet, P Dubreuil, A Moussy, KA Hahn.

SUMMARY
Constitutively active forms of c-Kit (a tyrosine kinase receptor regulating mast cell proliferation) carrying juxtamembrane (JM) mutations are expressed in 25% of canine mast cell tumours (MCT) and associated with higher tumour grade and worse prognosis.

Masitinib, a tyrosine kinase inhibitor targeting both wild type (WT) and JM c-Kit was tested for the treatment of recurrent or non-resectable grade 2/3 cutaneous MCT in a multicenter, randomized, placebo-controlled, double-blind study. Endpoints were tumour response/progression (WHO criteria) and overall survival (OS). We previously reported that masitinib was effective in the treatment of MCT in the overall population of dogs. The present report focuses on 139 dogs with tumours expressing WT c-Kit only.

Dogs received either masitinib (12.5 mg/kg/day, per os) or placebo. This masitinib dosage resulted in blood concentrations (Cmin: 100nM – Cmax: 2μM) above the IC50 for WT c-Kit inhibition (100nM) in pharmacokinetic studies and subgroup efficacy analyses showed that masitinib could be efficient on tumours expressing WT c-Kit.

When used on non-resectable tumours, masitinib significantly improved time-to-progression (TTP) with a median of 140 days (versus 75 days under placebo, Log-Rank p=0.025, hazard ratio=1.93). At 12-months, 28.9% of dogs had controlled disease (versus 0% under placebo, p=0.015) and 11.5% of dogs were in complete remission. Moreover, at 24-months, 6.7% of dogs were still in complete remission. There was a trend for improved survival. Masitinib doubled survival time with a median survival of 779 days (versus 361 days under placebo) and increased survival rate at 12-months (60.7% versus 43.8% under placebo) and 24-months (39.2% versus 23.1% under placebo).

When used as first-line therapy, masitinib significantly improved TTP with a median of 253 days (versus 66 days under placebo, Log-Rank p=0.008, hazard ratio=2.74). At 12-months, 36.4% of dogs had controlled disease (versus 0% under placebo, p=0.04) and 9.1% of dogs were in complete remission. Moreover, at 24-months, 7.1% of dogs were still in complete remission. There was a trend for improved survival. Masitinib tripled survival time with a median survival of 937 days (compared to 286 days under placebo) and increased survival rate at 12-months (69.4% versus 44.4% under placebo) and 24-months (54.8% versus 25.0% under placebo).

In conclusion, masitinib improved tumour control and survival and is therefore indicated in the treatment of MCT expressing WT c-Kit, especially when used on non-resectable tumours or as first-line therapy.
MASITINIB REDUCES THE ONSET OF METASTASIS AND IMPROVES LONG-TERM SURVIVAL IN DOGS WITH MEASURABLE GRADE 2 AND GRADE 3 MAST CELL TUMOURS.

Publication Category: Conference Presentation

Proceedings of the British Small Animal Veterinary Association Congress, 2009
O Hermine, JP Kinet, P Dubreuil, A Moussy, KA Hahn.

SUMMARY

We evaluated masitinib for the treatment of canine grade 2/3 mast cell tumours (MCT) in multicenter, randomized, placebo-controlled, double-blind study. Previously reported data at 6-months showed that masitinib significantly improved time-to-progression (Log-Rank p=0.033). Here we report data on metastases and follow-up at 24-months. Two-hundred-and-two dogs having recurrent or non-resectable grade 2/3 cutaneous MCT without lymph node or visceral metastases received either masitinib (12.5 mg/kg/day, per os, 161 dogs) or placebo (41 dogs). After 6-months, dogs could extend treatment with tri-monthly tumour assessments. Endpoints were tumour response/progression (WHO criteria) and overall survival (OS). Masitinib significantly reduced lymph node and visceral metastases. Only 3.7% of dogs under masitinib developed lymph node or visceral metastases (versus 17.1% of dogs under placebo; p<0.001).

Two-year follow-up data confirmed the improvement of time-to-progression and showed that masitinib induces long-lasting tumour control. At 12-months, 23.1% of dogs under masitinib had controlled disease (compared to 5.9% of dogs under placebo, p=0.026) and 11.1% were in complete remission. Moreover, 8.3% remained in complete remission at 24-months.

Masitinib was especially efficient on non-resectable tumours. At 12-month, 31.6% of dogs under masitinib had controlled disease (versus no dogs under placebo, p<0.001) and 15.8% were in complete remission. Moreover, at 24-months, 11.8% of dogs remained in complete remission. Masitinib also improved survival. It increased survival rate at 12-months (61.3% versus 37.5% under placebo, p=0.041) and at 24-months (36.4% versus 15.0% under placebo) and almost doubled the survival time with a median survival of 617 days (versus 322 days under placebo).

Masitinib was also particularly efficient as a first-line therapy. At 12-months, 39.6% of dogs under masitinib had controlled disease (versus no dogs under placebo, p=0.002) and 14.6% were in complete remission. Moreover, at 24-months, 14.3% of dogs remained in complete remission. Masitinib significantly increased survival rate at 12-months (67.9% versus 37.5% under placebo, p=0.042) and 24-months (48.9% versus 14.3% under placebo, p=0.03) and doubled survival time with a median survival of 823 days (versus 322 days under placebo, Log-Rank p=0.021).

In conclusion, masitinib significantly reduced the development of lymph node and visceral metastases, induced a long-lasting control of tumour progression and was curative (complete remission at 24-months) in a subset of patients. When masitinib was used as a first line treatment and/or on non-resectable tumours, it was particularly efficient and significantly improved long-term survival.
5.15 A NOVEL C-KIT INHIBITOR (AB1010) SHOWS THERAPEUTIC POTENTIAL IN DOG MAST CELL TUMORS (DMCT)

Publication Category: Conference Presentation

Proceedings of the 26th Annual Conference of the Veterinary Cancer Society, 2006

SUMMARY
AB1010 is an orally available small molecule that inhibits the juxtamembrane (JM) mutations of c-Kit with a 10-9M IC50. These mutations are associated with the development of DMCT.

An open-labeled Phase II study was performed to determine the therapeutic potential of AB1010. Dogs having measurable cutaneous grade 2 or 3 tumors, with or without metastasis, were evaluated. Thirteen dogs were enrolled with 9 dogs having sufficient treatment duration to evaluate for responsiveness to AB1010. Complete response was observed in 2 of 9 dogs, partial response in 2 of 9 dogs, stable response in 2 of 9 dogs, with 3 dogs having progressive disease. A partial response of the metastatic disease was observed in 2 of 3 dogs. Measurable responses were noted as early as day 7 of the study period. One of the 2 complete responders maintained remission for at least 182 days. The drug was well tolerated and grade 1 neutropenia was occasionally observed without consequences.

A randomized, double blinded, placebo-controlled, Phase III study is on going to confirm the clinical efficacy of AB1010. Main criteria for inclusion are: (1) non-resectable tumors or recurrent tumors following surgery (2) histopathologic grade 2 or 3 (3) no internal metastasis or lymph node involvement. To date, 170 dogs have been recruited. Complete results will be available in 2007. Genotyping of 40 tumors has been accomplished. Results show c-Kit expression in 100% of the tumors with less than 50% expressing the JM mutations. Furthermore preliminary clinical data strongly suggest that WT-c-Kit expressing tumors are also sensitive to AB1010. Taken together, our data indicate that other oncogenic mechanisms must be involved in the formation of DMCT, some of which appear to be sensitive to AB1010.
INTRODUCTION: Masitinib is an oral tyrosine kinase inhibitor that selectively targets c-Kit and PDGFR. In-vitro studies have shown that T-cell lymphoma cells are particularly sensitive to the anti-proliferative action of masitinib. In humans, mycosis fungoides has shown positive immunohistochemistry reaction for both PDGF and PDGFR in the abnormal cells.

MATERIAL AND METHODS: A 10.5 year-old Dachshund, female with diagnosed T-cell epitheliotropic lymphoma showed relapse with severe skin inflammation, erythema, alopecia and pruritus after COP chemotherapy (3 months partial response), and lomustine with prednisone (3 months partial response). Masitinib was administered in monotherapy at a dose of 12.5 mg/kg/day.

RESULTS: Following 1 week of masitinib treatment a significant improvement in skin and general condition was observed, with a complete response documented after 3 weeks. Throughout the duration of treatment an improvement in the patient’s quality of life was also observed. After 4 months the patient was lost to follow-up due to death, likely due to anemia without clear causality. No postmortem examination was performed. Immunohistochemistry on the biopsy showed intense staining for phosphorylated PDGFR and discrete staining for PDGFR, consistent with human findings.

CONCLUSIONS: Tumor cell-derived PDGF may play an important role in tumorigenesis. Hence, selective inhibition of PDGFR with subsequent apoptosis of abnormal cells might explain the observed positive response to masitinib. Together with supporting in-vitro findings this suggests that masitinib might represent a therapeutic option for dogs with T-cell epitheliotropic lymphoma. Further study is required to classify the morphological type of the neoplastic lymphocytes, and to assess treatment efficacy in a larger study cohort.
6.2 EVALUATION OF THE RECEPTOR TYROSINE KINASE INHIBITOR, MASITINIB MESYLATE, IN CANINE OSTEOSARCOMA CELL LINES

Publication Category: Conference Presentation

Proceedings of the 31st Annual Conference of the Veterinary Cancer Society, 4-7 November, 2011.
Fahey C, Kow K, Milner R, Salute M

INTRODUCTION: Osteosarcoma (OSA) cells have been shown to express flk-1 (VEGFR2), constitutively activated PDGFR-beta, and anti-apoptotic factors such as bc1-2. In dogs with OSA, increased levels of vascular endothelial growth factor (VEGF) have been documented, suggesting a mechanism for metastatic disease. Masitinib mesylate is a tyrosine kinase inhibitor that selectively targets c-Kit and PDGFR-alpha/beta, as well as other tyrosine kinases. The purpose of this study was to determine the IC50 of masitinib in OSA cells, its ability to induce apoptosis in OSA cells, and its capacity to modulate VEGF expression when these cells are exposed to masitinib.

METHODS: Three osteosarcoma cell lines (POS, HMPOS, COS31) were treated with increasing concentrations of masitinib mesylate (0.1 µM - 50µM) for 24, 48, and 72 hours. The IC50 was determined using a Cell Titer Blue® cell viability assay. Apoptosis was measured using the Apo-ONE® Homogeneous caspase-3/7 assay and verified using a TUNEL assay. Levels of VEGF at 72 hours were evaluated using a Quantikine® canine VEGF immunoassay.

RESULTS: The IC50 at 72 hours for POS, HMPOS and COS31 were determined to be 11.04 µM, 7.09 µM, and 9.74 µM, respectively. Apoptosis was shown to increase in correlation with the IC50 consistently in each cell line. VEGF levels increased in close proximity to the IC50 of each cell line.

CONCLUSIONS: Masitinib mesylate causes dose-dependent osteosarcoma cell death. One mechanism of cell death due to masitinib mesylate is caspase mediated apoptosis. The significance of elevations of VEGF prior to or concurrently with apoptosis and cell death requires further investigation.
6.3 MASITINIB FOR MAINTENANCE CHEMOTHERAPY OF TWO DOGS WITH T-CELL MULTICENTRIC LYMPHOMA

Publication Category: Conference Presentation

Proceedings of the Annual Congress of the European Society of Veterinary Oncology, March 18-20, 2010
F Serres, C Meyer, D Tierny, A Hidalgo, C Haelewyn, L Marescaux

SUMMARY
Response to treatment of canine T-cell multicentric lymphoma is classically described as poor, especially for pleomorphic and lymphoblastic forms, with median survival times of less than 8 months in most studies focusing on the interest of «conventional» chemotherapy. In vitro studies have shown that canine T-cell might be sensitive to masitinib, owing to the inhibition of c-Kit/PDGFRα&β.

Two Shih-tzu female dogs were presented for clinical staging and evaluation of therapeutic possibility in multicentric lymphoma. Dogs were clinically staged 3-a and cytologic examination of enlarged lymph nodes was suggestive of clear cell lymphoma (CCL) and large granular lymphoma (LGL), respectively. Immunostaining was positive for CD-3 and negative for CD-79, indicating a T-Cell origin. Both dogs had been previously treated with glucocorticoids. The dog with CCL initially received vincristin (as part of a CHOP based protocol), but treatment was poorly tolerated and the owner withdrew consent prior to the induction period. The dog with LGL received one initial administration of L-asparaginase. Both dogs received masitinib (12 and 12,2 mg/kg/day, respectively) after this initial induction period.

Masitinib was well tolerated (clinically and according to haematology, serum biochemistry and urinanalysis). Progressive complete remission was reached in the dog with CCL, which remained present at the time of writing, 8 months after initial diagnosis. Rapid complete remission was reached in the dog with LGL, which remained present at the time of writing, 3 months after initial diagnosis.

Masitinib was well tolerated and could represent a therapeutic option in dogs with T-Cell multicentric lymphoma.
A C-KIT INHIBITOR (MASIVET®) SHOWS THERAPEUTIC POTENTIAL IN DOG NEUROFIBROSARCOMA

Publication Category: Conference Presentation

Proceedings of the Annual Congress of the European Society of Veterinary Oncology, March 18-20, 2010
A Roos, S Fraytag, A. Moussy, O. Hermine.

BACKGROUND
An 11 year-old, mixed breed, female dog was diagnosed with neurofibrosarcoma in October 2007. The tumour recurred despite two prior surgeries, chemotherapy with doxorubicine and metronomic chemotherapy (endoxan/meloxicam). A strong relationship exists between the pathogenesis of neurofibrosarcoma and both mast cells and Schwann cells. This suggests possible benefits from treatment with targeted c-Kit or PDGFR inhibitors, respectively. Masitinib (Masivet®) is a tyrosine kinase inhibitor (TKI) that potently and selectively inhibits PDGFR and c-Kit, as well as Lyn and Fyn, without inhibiting kinases of known toxicities.

METHODS
Neurofibrosarcoma was diagnosed by histological analysis. Mast cell infiltration was evidenced by c-Kit and tryptase staining. Treatment with Masivet® was initiated in April 2009 with 12 mg/kg/day administered orally. No concomitant treatments were administered and the patient was careful monitored for adverse events.

RESULTS
The tumour was infiltrated by scattered mast cells at the vicinity of blood vessels. Signs of superficial necrosis were visible at day-4 of treatment. At week-3 a reference mass was measured to be 5x4cm, which at week-6 had shrunk to 3x2cm and was negligible at week-10. However, new masses may indicate a higher dosage is required to control tumour metastases. No adverse events were reported, with only a minor decrease in protein level.

CONCLUSION
This represents the first reported case of canine neurofibrosarcoma being treated with the TKI Masivet®. Rapid tumour growth was quickly stabilised followed by a significant decrease in volume; treatment was well tolerated. There is compelling motivation to conduct further preclinical (mechanistic, resistance) and clinical (efficacy and safety) studies.
6.5 EVALUATION OF THE RECEPTOR TYROSINE KINASE INHIBITOR, MASITINIB MESYLATE, IN CANINE HEMANGIOSARCOMA CELL LINES

Publication Category: Conference Presentation

Proceedings of the 30th Annual Conference of the Veterinary Cancer Society, October 29-November 1, 2010 (also in Veterinary and Comparative Oncology, 9, 1, e1–e49. DOI: 10.1111/j.1476-5829.2010.00252.x)
S. Lyles, R. Milner, K. Kow and M. Salute

INTRODUCTION: Haemangiosarcoma (HSA) cells have been shown to express c-kit (CD117), flk-1 (VEGFR2), constitutively activated platelet-derived growth factor receptor-β (PDGFR-β) and anti-apoptotic factors such as bcl-2 and survivin. In dogs with HSA, increased levels of vascular endothelial growth factor (VEGF) have been documented, suggesting a mechanism for early metastatic disease. Masitinib mesylate is a tyrosine kinase inhibitor that selectively targets c-kit and PDGFR-α/β, as well as other tyrosine kinases.

The purpose of this study was to determine the IC50 of masitinib in HSA cells, investigate masitinib’s ability to induce apoptosis in HSA cells and finally its capacity to modulate VEGF expression when these cells are exposed to masitinib.

METHODS: Three HSA cell lines (DEN, Fitz and SB) were treated with increasing concentrations of masitinib mesylate (0.01–100 μM) for 24, 48 and 72 h. The IC50 was determined using a Cell Titer Blue® cell viability assay. Apoptosis was measured using the Apo-ONE® Homogeneous caspase-3/7 assay. Levels of VEGF at 72 h were evaluated using a Quantikine® canine VEGF immunoassay.

RESULTS: The IC50 for DEN, Fitz and SB were determined to be 8.56, 9.41 and 10.65 μM, respectively. Apoptosis was shown to increase in correlation with the IC50 consistently in each cell line. VEGF levels increased in close proximity to the IC50 of each cell line.

CONCLUSIONS: Masitinib mesylate causes dose-dependent HSA cell death. One mechanism of cell death due to masitinib mesylate is caspase-mediated apoptosis. The significance of elevations of VEGF prior or concurrently with apoptosis and cell death requires further investigation.
6.6 MASITINIB, ORAL TYROSINE KINASE INHIBITOR, IN THE TREATMENT OF ADVANCED MELANOMA: PRECLINICAL PROOF-OF-CONCEPT AND PHASE 1/2/3 STUDY DESIGNS.

Publication Category: Conference Presentation

O. Hermine, Douglas Thamm, M Humbert, S Letard, A. Moussy, Patrice Dubreuil

SUMMARY

The continuing poor prognosis and lack of effective treatments for non-resectable or metastatic stage 3/4 melanoma highlight an unmet medical need in human and veterinary medicine. It is crucial therefore, to identify alternative or complementary treatment strategies that improve the clinical management and prognosis of patients afflicted with this cancer.

Masitinib is an oral tyrosine kinase inhibitor (TKI) that in vitro has greater activity and selectivity against c-Kit than imatinib. It is particularly efficient in controlling the proliferation, differentiation and degranulation of mast cells; cells that are involved in several mechanisms that facilitate tumour growth. Masitinib also potently targets PDGFR, Lyn and to a lesser extent the FAK pathway, without inhibiting kinases of known toxicities. Respectively, these kinases are associated with: modulating tumoural interstitial pressure and thus, chemotherapy uptake; regulation of cell signalling, adhesion, migration, apoptosis, cell cycle progression and resistance to conventional therapies; and the transduction pathway leading to mast cell IgE induced degranulation. Moreover, in vivo and in vitro studies have shown that masitinib can enhance the antiproliferative effects of chemotherapeutic agents to various human and canine cancer cell lines.

Specific melanomas have been shown to bear c-Kit aberrations (either mutation or amplification), strongly suggesting that a c-Kit inhibitor such as masitinib could be of therapeutic benefit to this subpopulation. Moreover, there is evidence that this targeted therapy can exert an anticancer action that extends beyond its inherent TKI profile, i.e. through the reduction of tumour progression and/or improved drug delivery and/or the inhibition of mast cell migration and activation.

We investigated masitinib’s potential to sensitize canine melanoma cell lines to chemotherapies using in vitro proliferation assays. Masitinib sensitized doxorubicin-sensitive (CML-6M and CML-10C2) cells, as well as resistant (17CM98) melanoma cells to doxorubicin (≥6-fold reduction in IC$_{50}$ at 5 to 10 µM masitinib). These data establish proof-of-concept that masitinib can sensitize canine melanoma tumour cell lines to doxorubicin; as well as cisplatin and gemcitabine to a lower extent in CML-6M. Further confirmation of masitinib’s therapeutic potential for this cancer is observed in veterinarian case studies, including a dog with histologically proven metastatic melanoma that was unresponsive to chemotherapy but showed a complete response after introduction of masitinib.

Based upon these studies (as well as successful clinical trials for masitinib treatment of canine MCT, human GIST and advance pancreatic cancer in combination with gemcitabine) two human clinical trials of masitinib treatment for non-resectable or metastatic stage 3/4 melanoma will be initiated in 2010. The first is a prospective, multicentre, open-label, uncontrolled, phase 1/2 study to evaluate masitinib in combination with dacarbazine in the treatment of patients not carrying a mutation in the juxta membrane domain of c-Kit. The second is a multicentre, randomised, open-label, active-controlled, two-parallel groups, phase 3 study to compare masitinib to dacarbazine in the treatment of patients carrying a mutation in the juxta membrane domain of c-Kit.
6.7 THE CYTOTOXIC EFFECT OF MASITINIB (MASIVET8) ON A CANINE LEUKEMIA CELL LINE DOES NOT RESULT FROM INHIBITION OF THE ABC-TRANSPORTERS P-GP AND MRP-1

Publication Category: Conference Presentation

Maurice Zandvliet, Erik Teske, Johanna Fink-Gienimels, Jan Schrickx

INTRODUCTION: Tumour cell resistance to chemotherapeutic agents is the main reason for treatment failure in canine malignant lymphoma. Resistance to doxorubicin, one of most potent chemotherapeutic agents available, is associated with resistance to other chemotherapeutic agents, a phenomenon known as Multi-Drug Resistance (MDR). One of the possible mechanisms of MDR is overexpression of drug-transporters of the ATP-Binding Cassette superfamily. In vitro work with masitinib, a tyrosine kinase inhibitor licensed for the treatment of mast cell tumours, showed that this drug has a cytotoxic effect on lymphoblasts and the ability to reverse MDR in multiple cell lines. As a result masitinib could potentially have a role in the treatment of dogs with MDR lymphoma, The underlying mechanism for reversal of MDR is unknown and the authors hypothesized that this is the result of inhibition of ABC-transporters.

MATERIALS AND METHODS: The GL-1 cell line, a canine leukemia cell line, was transformed into a doxorubicin-resistant subline (GL-40) by continuous incubation with gradually increasing concentrations of doxorubicin. By measuring retention of the substrates rhodamine123, calcein-AM and CFDA, the effect of increasing concentrations of masitinib was tested on Pgp and MRP-1 function in both GL-1 and GL-40. Cellular retention of fluorescent substrates and doxorubicin was analysed by FACS after 30 minutes of incubation with or without inhibitors.

RESULTS: At higher concentrations masitinib has a cytotoxic effect on the canine leukemia cell lines GL-1 and GL-40. Masitinib is an inhibitor of both P-GP and MRP-1 in GL-40. Inhibition of these ABC-transporters did not result in increased intracellular doxorubicin concentrations.

DISCUSSION: Masitinib is cytotoxic to canine leukemia cells. Although masitinib has an inhibitory effect on P-gp and MRP activity, this does not result in an increased intracellular doxorubicin retention. Therefore masitinib does not reverse MDR by inhibition of these ABC-transporters. Potential mechanisms to explain the observed results require additional studies.
INTRODUCTION: In vitro, masitinib sensitizes several canine tumor cell lines to doxorubicin. A phase I clinical study evaluated the safety of masitinib + doxorubicin in dogs with advanced tumors.

METHODS: Dogs first received masitinib (12.5 mg/kg/day PO) for 2 weeks; the masitinib was continued concurrently with at least two cycles of doxorubicin (one cycle=3 weeks). Successive cohorts of three dogs were enrolled. The first cohort received masitinib + doxorubicin (25 mg/m2 for dogs =15 kg or 0.8 mg/kg for dogs <15 kg, q3w). Doxorubicin dose escalation was considered for successive cohorts using a modified Fibonacci dose escalation scheme.

RESULTS: One dog was withdrawn for a renal adverse event before receiving doxorubicin and sixteen received masitinib + doxorubicin: eight dropped out prematurely for disease progression, six completed the study and two received higher than intended doses of doxorubicin. Three dogs received masitinib + doxorubicin (25 mg/m2 for dogs =15 kg or 0.8 mg/kg for dogs <15 kg, q3w). All completed two cycles without experiencing dose limiting toxicity (DLT). Three dogs received masitinib + doxorubicin (30 mg/m2 for dogs =15 kg or 1 mg/kg for dogs <15 kg, q3w). Two dogs completed two cycles without experiencing DLTs and one experienced a DLT (neutropenia). Two dogs (<15 kg) received higher than intended doses of doxorubicin (1.28 and 1.17 mg/ kg q3w, respectively). Both experienced DLTs.

CONCLUSIONS: The maximum tolerated dose of doxorubicin in combination with masitinib (12.5 mg/kg/day) was at least 30 mg/m2 for dogs =15 kg and 1 mg/kg for dogs <15 kg.
6.9 EVALUATION OF SAFETY OF MASITINIB IN COMBINATION WITH CARBOPLATIN IN ADVANCED CANINE TUMORS

Publication Category: Conference Presentation

Greg Ogilvie, Anthony Rusk, Alfred Legendre, Alain Moussy, Olivier Hermine

INTRODUCTION: Preclinical data suggest that masitinib sensitizes several canine tumor cell lines to carboplatin. A phase I clinical study evaluated the safety of masitinib associated with carboplatin in dogs with advanced tumors.

METHODS: Dogs first received masitinib (12.5 mg/kg/day PO) for 2 weeks; the masitinib was subsequently continued and given concurrently with at least two cycles of carboplatin (one cycle=3 weeks). Successive cohorts of three dogs were enrolled. The first cohort received masitinib + carboplatin (250 mg/m2 IV, q3w). Carboplatin dose escalation was considered for successive cohorts using a modified Fibonacci dose escalation scheme.

RESULTS: Six dogs with advanced tumors were recruited and received masitinib + carboplatin. Four dogs completed the study and two were withdrawn due to disease progression. The first three dogs received masitinib + carboplatin (250 mg/m2 IV q3w). Two dogs completed the two cycles without experiencing any dose-limiting toxicity (DLT) and one died because of disease progression during the third week of treatment. The next three dogs received masitinib + carboplatin (300 mg/m2 IV q3w). Two dogs completed the two cycles without experiencing any DLT and one dog had disease progression before receiving combination therapy. This dog received the first cycle of carboplatin but discontinued treatment before completing the two cycles because of disease progression.

CONCLUSIONS: For each cohort, two out of three dogs completed the two cycles of carboplatin in combination with masitinib without experiencing DLT. Therefore, the maximum tolerated dose of carboplatin in combination with masitinib (12.5 mg/kg/day) was at least 300 mg/m2.
7 CANINE ATOPIC DERMATITIS

7.1 MASITINIB DECREASES SIGNS OF CANINE ATOPIC DERMATITIS: A MULTICENTRE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE 3 TRIAL’

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)


SUMMARY
This study investigated the efficacy and safety of masitinib, a selective tyrosine kinase inhibitor capable of downregulating mast cell functions, for treatment of canine atopic dermatitis (CAD). Dogs with confirmed CAD received masitinib at 12.5 mg/kg/day (n = 202) or control (n = 104) for 12 weeks. A reduction in CAD 3 Extent and Severity Index (CADESI-02) score of 50% at week 12 was observed in 61% of masitinib-treated dogs versus 35% of control dogs (P < 0.001), according to the modified intent-to-treat population, observed cases data set (quoted hereafter unless stated otherwise). The corresponding result according to the missing considered as failure data set was 43 versus 26%, respectively (P < 0.001). For dogs resistant to ciclosporin and/or corticosteroids (60% of the study population), CADESI-02 response rates were 60 versus 31%, respectively (P = 0.004). The mean reduction in pruritus score of severely pruritic dogs was 46 versus 29%, respectively (P = 0.045). Furthermore, 65% of owners with severely pruritic dogs assessed masitinib efficacy as good/excellent versus 35% control (P = 0.05). Overall, 63% of investigators assessed masitinib efficacy as good/excellent versus 35% control (P < 0.001). Premature discontinuations from the modified (intent-to-treat) population (28.2% masitinib versus 26.0% control) were mainly due to adverse events (13.4 versus 4.8%, respectively) or lack of efficacy (12.4 versus 18.3%, respectively). In total, 13.2% dogs presented with severe adverse events (16.0% masitinib versus 7.7% control). Masitinib showed a risk of reversible protein loss, although regular surveillance of blood albumin and proteinuria allowed 4 for discontinuation of treatment while the dog was still clinically asymptomatic. Masitinib proved to be an effective and mostly well-tolerated treatment of CAD, including severe and refractory cases, with medically manageable adverse effects.
INTRODUCTION: Masitinib is a tyrosine kinase inhibitor that potently and selectively targets c-Kit and Lyn, therefore, down-regulating mast cell functions.

METHODS: Randomized (2:1), double-blinded trial, 44 centers in Europe and the USA. Dogs with a confirmed diagnosis of CAD (CADESI-02 ≥25), regardless of pruritus score, were eligible. Dogs received masitinib (12.5mg/kg/day) or placebo for 12 weeks with concomitant flea treatment. A restrictive list of prescribed antimicrobial/antifungal/antiseptic treatments was permitted.

RESULTS: A reduction in CADESI-02 of >50% was observed in 61% of masitinib-treated dogs (N=202) vs. 35% placebo (N=104); (p<0.001, observed cases at W12 [OC]). For dogs resistant to cyclosporine and/or corticosteroids (60% of population), CADESI-02 response rates were 60% vs. 31%, respectively (p=0.004, OC). The mean reduction in pruritus score of severely pruritic dogs was 46% vs. 29%, respectively (p=0.045, OC), with the related CADESI-02 excoriation sub-score showing improvement for masitinib and deterioration in placebo (-34% vs. +35%, respectively). Furthermore, 65% of owners with such dogs assessed masitinib efficacy as good/excellent vs. 35% placebo (p=0.05, OC). Overall, 63% of investigators assessed masitinib efficacy as good/excellent vs. 35% placebo (p<0.001, OC). Concomitant treatments with antimicrobials and/or antiseptics were required more frequently in the placebo group (p=0.055). Premature discontinuations (28.2% masitinib vs. 26.0% placebo) were mainly due to adverse events (13.3% vs. 4.8%, respectively) or lack of efficacy (12.4% vs. 18.3%, respectively). In total, 15.2% dogs presented with severe adverse events (18.0% masitinib vs. 9.6% placebo), including 1.9% deaths (1.4% vs. 2.9%, respectively).

CONCLUSIONS: Masitinib proved to be an effective treatment of CAD, even in dogs resistant to current therapies, with medically manageable side-effects.
7.3 THE TYROSINE KINASE INHIBITOR MASITINIB (MASIVET®) IS EFFECTIVE FOR TREATMENT OF ATOPIC DERMATITIS IN DOGS

Publication Category: Conference Presentation

New Trends in Allergy VII Congress. Special Workshop: Atopic Dermatitis in Dogs, 2010

SUMMARY
The long term treatment of canine atopic dermatitis (CAD) remains an unmet medical need, especially in severe and refractory cases. Mast cells (MC) 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, MCs represent an attractive, hitherto untapped, therapeutic target for CAD.

Masitinib is a tyrosine kinase inhibitor that potently and selectively targets c-Kit and Lyn, and consequently down-regulates MC functions. Proof-of-concept that masitinib can safely reduce symptoms associated with inflammatory diseases in veterinary and human medicines have been demonstrated via Phase 1/2 studies (e.g. human asthma, arthritis, psoriasis; feline asthma and CAD).

Reported are results from a randomised, controlled, pivotal phase 3 trial, to compare efficacy and safety of masitinib at 12.5 mg/kg/d to placebo in the treatment of CAD over 12 weeks. Dogs with a confirmed diagnosis of CAD (CADESI-02 ≥25), regardless of pruritus score, were eligible. A reduction in CADESI-02 of >50% was observed in 61% of masitinib-treated dogs (N=202) vs. 35% placebo (N=104); (p<0.001, observed cases at W12 [OC]). For dogs resistant to cyclosporine and/or corticosteroids (60% of population), CADESI-02 response rates were 60% vs. 31%, respectively (p=0.004, OC). The mean reduction in pruritus score of severely pruritic dogs was 46% vs. 29%, respectively (p=0.045, OC). Furthermore, 65% of owners with such dogs assessed masitinib efficacy as good/excellent vs. 35% placebo (p=0.05, OC). Overall, 63% of investigators assessed masitinib efficacy as good/excellent vs. 35% placebo (p<0.001, OC). In total, 15.2% dogs presented with severe adverse events (18.0% masitinib vs. 9.6% placebo), including 1.9% deaths (1.4% vs. 2.9%, respectively).

Masitinib proved to be an effective and safe treatment of CAD, even in dogs resistant to current therapies, with medically manageable side-effects.
7.4 MASITINIB FOR THE TREATMENT OF CANINE ATOPIC DERMATITIS: A PILOT STUDY

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)

Daigle J, Moussy A, Mansfield CD, Hermine O.

SUMMARY
There is an on-going need to identify medications suitable for the long-term treatment of canine atopic dermatitis (CAD). Masitinib mesilate is a potent and selective tyrosine kinase inhibitor of the c-KIT receptor. A strong relationship exists between the SCF/c-KIT pathway and pathogenesis of CAD, suggesting that masitinib may potentially fulfil the above role. This study reports on an uncontrolled pilot study of masitinib in CAD. Masitinib was administered orally to 11 dogs at a mean dose of 11.0 +/- 1.83 mg/kg/day (free base) for 28 days. Treatment response was assessed by evolution of clinical appearance according to a modified version of the Canine Atopic Dermatitis Extent and Severity Index (mCADESI), pruritus scale and surface area of lesions. Masitinib improved CAD with a mean reduction in mCADESI of 50.7 +/- 29.8% (95% C.I. = 29.4-72.0; p = 0.0004) at day 28 relative to baseline, with 8/10, 8/10 and 4/10 dogs showing improvement of >or=33%, >or=40% and >or=50%, respectively. Improvement was further evidenced by a decrease in pruritus score and the surface area of lesions. No serious or severe adverse events occurred during this trial, although 6/11 dogs presented with mild to moderate treatment related adverse events. There is sufficient compelling evidence to warrant further investigation.
7.5 EFFICACY AND SAFETY OF MASITINIB ON THE TREATMENT OF ATOPIC DERMATITIS IN DOGS

Publication Category: Conference Presentation


SUMMARY
We previously described masitinib, a protein-tyrosine kinase inhibitor targeting c-Kit, the receptor for stem cell factor, which is a key regulator of mast cell differentiation and proliferation. Mast cells are well known to participate in the pathophysiology of atopic dermatitis, a very common disease in dogs. Therefore, in the current study, we explored the efficacy of masitinib in treating canine atopic dermatitis.

This was a Phase 2 non-controlled pilot study performed in two centers. The study including a total of 11 dogs (6 male, 5 female) between 0.9 and 7.9 years of age presenting with atopic dermatitis according to Prelaud’s criteria. The dogs received daily oral treatment for 4 weeks with 10 mg/kg/day of masitinib free base, with dose adjustments according to efficacy and appearance of toxicity. The canine atopic dermatitis extent and severity index (CADESI) and the lesion surface (% of body area) were determined on days 0, 14, and 28, a pruritus score (0 to 4) was calculated on days 0 and 28, and toxicity was assessed by blood tests on days 14 and 28 as well as by clinical observations.

The mean total CADESI improved by 38.3 ± 29.2% (P=0.0025 [n=10]) and 50.7 ± 29.8% (P=0.0025 [n=10]) at days 14 and 28, respectively. An improvement ≥ 33%, 40%, and 50% was observed in 8/10, 8/10, and 4/10 of the patients, respectively. CADESI subscores for erythema (19.0 ± 15.2 vs 34.7 ± 12.5 [P=0.005]), lichenification (2.6 ± 2.5 vs 9.3 ± 9.8 [P=0.0371]), excoriati (2.9 ± 2.4 vs 9.9 ± 7.0 [P=0.0177]), and scraping alopecia (10.5 ± 13.6 vs 22.6 ± 22.9 [P=0.0177]) were significantly improved at day 28, although the subscore of papules did not change (10.1 ± 11.3 vs 11.3 ± 12.3 [P=0.5086]). Between days 0 and 28, the change in pruritus score was −2 for 3/10, −1 for 2/10, 0 for 3/10, and +1 for 2/10 patients. In 4 of the 5 patients with unchanged or increased pruritus score, the total CADESI decreased. In addition, there was a decrease in the surface area of lesions between days 0 and 28. Ten adverse events were reported in 7 of the 11 dogs, and there were no severe adverse events. Only one adverse event associated with the study drug (febrile episode lasting 2 days) led to treatment arrest, and it resolved without sequelae. An expected decrease of white blood cells related to the effect of the study drug was observed, although, when present, neutropenia was of mild or moderate severity.

These results show that the treatment was safe and effective for canine atopic dermatitis. Accordingly, a Phase 3 study to confirm these findings is warranted.
8 TREATMENT OF FELINE CONDITIONS

8.1 MASITINIB DEMONSTRATES ANTI-PROLIFERATIVE AND PRO-APOPTOTIC ACTIVITY IN PRIMARY AND METASTATIC FELINE INJECTION-SITE SARCOMA CELLS

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)

Lawrence, J., Saba, C., Gogal, R., Lamberth, O., Vandenplas, M. L., Hurley, D. J., Dubreuil, P., Hermine, O., Dobbin, K. and Turek, M

SUMMARY
Dysregulation of platelet-derived growth factor receptor (PDGFR) may play a role in feline injection site sarcoma (ISS) cell growth and viability. Masitinib, a tyrosine kinase inhibitor approved for treatment of canine mast cell tumors, is highly selective for the PDGFR signaling pathway and may offer a new therapeutic approach for this disease. The in vitro effects of masitinib on growth, induction of apoptosis, and activation of PDGFR signaling in two novel feline ISS cell lines were investigated. PDGFR expression was confirmed by Western blot in cell lines derived from a primary ISS tumor (JB) and a corresponding, histologically-confirmed ISS lung metastasis (JBLM). Masitinib inhibited cell growth and PDGFR tyrosine phosphorylation in both cell lines. Drug concentrations required to inhibit growth were higher than those required to modulate ligand-induced autophosphorylation of the PDGFR. These in vitro data suggest that masitinib displays activity against both a primary and metastatic ISS cell line and may have a role in the clinical management of this disease.
8.2 IN VITRO INVESTIGATION OF THE USE OF MASITINIB AND RADIATION ON FELINE INJECTION SITE SARCOMA CELLS

Publication Category: Conference Presentation

Proceedings of the 2011 GVSP symposium, August 5-6.

SUMMARY
Injection site sarcoma (ISS) is a soft tissue tumor that can develop at the site where an inactivated vaccine has been administered, particularly rabies and feline leukemia virus vaccines. Current therapy for management of ISS includes a combination of surgery and radiation therapy. Platelet derived growth factor receptor (PDGFR) is a protein kinase that has been shown to be critical in ISS cell survival and signal transduction and interruption of PDGFR signaling may represent a potential therapeutic strategy for cats with ISS. Masitinib mesylate (AB Science, Paris, France), a tyrosine kinase inhibitor, is highly selective for the PDGFR signaling pathway. Our research group recently showed that masitinib inhibited cell growth and PDGFR phosphorylation in a primary (JB) and metastatic (JBLM) cell line.

As radiation is a conventional therapy used in clinical management of ISS, the combined in vitro effects of masitinib and radiation in JB and JBLM were investigated. The objective of this study was to determine if the radiosensitivity of ISS cells was altered in the presence of masitinib. Alamar Blue proliferation assays were used to generate dose-response curves following treatment with varying doses of masitinib (0-86 µM) and radiation (0-6 Gy). Masitinib and radiation demonstrated significant interactions with one another to negatively impact cell proliferation. Across a variety of radiation doses, masitinib showed a similar dose-dependent inhibitory effect in both cell lines. Additionally, prolonged exposure to single agent masitinib induced significant cell inhibition at doses < 0.1 µM, suggesting potential for chronic dosing regimens.

Parallel to proliferation assays, work has focused on examining the interactions between masitinib and radiation. There is strong evidence that DNA is the principal target of radiation, and the induction of DNA double strand breaks has been assessed with the use of immunocytochemistry for phosphorylated histone γH2AX. Using Western blot analysis and a commercially available ELISA (Millipore), we validated an antibody against γH2AX and found a significant increase in γH2AX in irradiated cells compared to control (unirradiated) cells. Current work is focused on determining if changes in γH2AX induction contribute to cell inhibition. Based on this preliminary data demonstrating a positive interaction between radiation and masitinib, additional in vitro investigation into the role of masitinib in the management of ISS is warranted.
8.3 EFFECT OF PLATELET-DERIVED GROWTH FACTOR RECEPTOR INHIBITION ON INJECTION-SITE SARCOMA RADIOSENSITIVITY

Publication Category: Conference Presentation

Hill J, Boullire B, Gogal Jr. RM, Lawrence J, Vandenplas M, Saba C, Turek M.

SUMMARY
Injection site sarcoma (ISS) has been an increasingly recognized malignancy in cats since it was first described in 1991. The correlation between vaccination and the development of sarcoma at vaccination sites is well documented. Feline ISS is locally invasive and, unless located distally on a limb, is best managed with a combination of surgery and radiation therapy. Despite the use of combination therapies, many cats with ISS will develop recurrence or metastasis, eventually resulting in death. Platelet derived growth factor receptor (PDGFR) is a protein kinase that has been shown to be important in ISS cell survival and signal transduction. Interruption of PDGFR signaling may represent a potential therapeutic strategy for cats with ISS. Masitinib mesylate (AB Science, Paris, France), a tyrosine kinase inhibitor, is highly selective for the PDGFR signaling pathway and is of interest in the management of this tumor. As radiation is a conventional therapy used in clinical management of ISS, the objective of this study is to determine if the radiosensitivity of ISS cells is altered in the presence of masitinib.

To examine the potential usefulness of combining masitinib with radiation therapy for rapidly dividing feline ISS, clonogenic assays were performed using an ISS cell line (KH) to determine the effects of masitinib and radiation on cell survival. ISS cells in monolayer culture were irradiated with a single radiation exposure (0, 3, 6, 9 Gray). Survival following radiation exposure was defined as the ability of the cells to maintain clonogenic capacity and to form colonies. Cells were plated for colony formation and colonies consisting of ≥50 cells were counted following incubation. Radiation experiments were carried out in control KH cells (without masitinib) and in KH cells exposed to 1 µM and 9 µM masitinib for 24 hours before radiation.

Preliminary results indicate that masitinib and radiation reduced KH cell survival in a radiation-dose-dependent fashion. Based on these preliminary data, additional in vitro investigation into the role of masitinib in the management of ISS is warranted.
8.4 SAFETY OF MASITINIB MESYLATED IN HEALTHY CATS

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)


BACKGROUND: Masitinib mesylate is a PO-administered tyrosine kinase inhibitor developed both for human and animal diseases with activity against both mutated and wild type forms of the c-kit receptor and platelet-derived growth factor receptors α and β, and is currently registered in Europe for the treatment of mast cell tumors in dogs.

HYPOTHESIS/OBJECTIVES: The objective of this study was to determine if healthy cats can tolerate administration of masitinib without clinically relevant adverse effects. Animals: Twenty healthy research colony-specific pathogen-free cats.

METHODS: This study was a prospective, randomized phase 1 clinical trial. Masitinib was administered PO to 20 healthy cats. Ten cats received 50 mg masitinib every other day for 4 weeks, and 10 cats received 50 mg masitinib daily for 4 weeks.

RESULTS: Clinically relevant proteinuria was noted in 2/20 (10%) cats (both treated daily), and neutropenia was noted in 3/20 (15%) (seen in both treatment groups). An increase in serum creatinine concentration and adverse gastrointestinal effects were noted in some cats.

CONCLUSIONS AND CLINICAL IMPORTANCE: Masitinib mesylate was tolerated in the majority of cats. Long-term administration and pharmacokinetic studies are needed to further assess the use of masitinib in cats.
8.5 PHARMACOKINETICS OF MASITINIB IN CATS

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)

Vet Res Commun 2009; 33:831–837
Bellamy F, Bader T, Moussy A, Hermine O.

SUMMARY
Masitinib is the first veterinary drug recently approved in Europe to treat mast cell tumours in dogs. This inhibitor is selective and highly efficient in blocking c-Kit, PDGFR, and Lyn tyrosine kinase activities. It showed good efficacy and acceptable toxicity in several animal studies such as mice, rats, rabbits and dogs. C-kit is a tyrosine kinase receptor that plays a critical role in the biology of mast cells including differentiation, survival, migration and cytokine/mediator release. Mast cells are involved in a number of allergy-and immune-related diseases in cats such as asthma, inflammatory bowel disease, and feline mast cell tumours. Therefore, there might be a strong rationale to use masitinib in these indications. Here, we report the results of a preliminary pharmacokinetic study of masitinib in cats which showed a good bioavailability of ~60% in both sexes. We propose that an oral dose of 10-15 mg/kg masitinib is appropriate to achieve adequate plasma concentrations.
SUMMARY
Tyrosine kinase inhibitors (TKI) represent a novel treatment for asthma. We hypothesized that the TKI masitinib would decrease airway inflammation and hyperreactivity with minimal toxicity in experimental feline allergic asthma.

Twelve asthmatic cats received 50mg/day masitinib or placebo orally for 12 weeks. Bronchoalveolar lavage fluid (BALF) % eosinophils and airway hyperreactivity in response to methacholine (MCh) challenge using ventilator acquired mechanics were determined. Results of MCh challenges were reported as the effective MCh concentration that increased baseline airway resistance by 200% (EC200Raw) and the end-inspiratory pressure after a breath hold (plateau pressure (Pplat)). Treatment was interrupted if predetermined adverse events were noted.

Baseline group mean±SD % BALF eosinophils were not significantly different between treatments (masitinib, 46±24%; placebo, 44±22%; p=0.345). After 4 weeks, the group mean±SD % BALF eosinophils was significantly lower in cats receiving masitinib compared with placebo (7±9% and 30±27%, respectively; p=0.023). There was no significant difference in the group mean±SD EC200Raw between groups at baseline or at 4 weeks. However, after 4 weeks, the Pplat with MCh challenge significantly (p=0.016) increased in placebo but not masitinib-treated cats (p=0.32). Masitinib-treated cats were significantly more likely to develop proteinuria (p=0.001) leading to drug interruption or withdrawal from study. Proteinuria was self-limiting in all cases after discontinuation of drug.

Masitinib at 50mg/cat/day significantly reduced eosinophilic airway inflammation and blunted one index of MCh-induced airflow limitation (Pplat) after 4 weeks. Adverse effects (specifically proteinuria), while reversible on discontinuation of drug, were common.
INTRODUCTION: Platelet derived growth factor receptor (PDGFR) has been shown to play a role in the growth and viability of vaccine-associated sarcoma (VAS) cells. Masitinib is a tyrosine kinase inhibitor that specifically targets multiple growth factor receptors including PDGFR. The objective of this study was to evaluate the in vitro effects of masitinib on VAS cell proliferation, apoptosis and PDGFR phosphorylation.

METHODS: VAS cell lines were derived at our institution from a primary tumor and a corresponding, histologically confirmed, VAS lung metastasis. PDGFR expression was confirmed by Western blot and flow cytometry (FACScan) analysis. Masitinib (0-100 AM, generously provided by AB Science) was co-cultured with each cell line to generate dose response curves measured by Alamar BlueTM cell viability/proliferation assays. Cellular apoptosis was assessed by 7-AAD flow cytometry. Masitinib-mediated suppression of PDGF-induced phosphorylation of PDGFR was evaluated by immunoprecipitation/Western blot assays. All experiments were performed in triplicate.

RESULTS: Masitinib resulted in a marked decrease in cell proliferation and an increase in apoptosis in a dose-dependent fashion. Growth inhibition and apoptosis correlated with the inhibition of PDGFR tyrosine phosphorylation in both cell lines.

CONCLUSIONS: These findings suggest that masitinib represents a growth-inhibitory agent that can influence cellular proliferation, apoptosis and PDGFR activation in VAS. This supports further investigation of masitinib as a novel therapeutic agent to treat VAS in cats.
8.8 EVALUATION OF THE RECEPTOR TYROSINE KINASE INHIBITOR, MASITINIB MESYLEATE, IN FELINE VACCINE ASSOCIATED SARCOMA CELL LINES AND HEALTHY CATS

Publication Category: Conference Presentation

Proceedings of the 29th Annual Conference of the Veterinary Cancer Society, October 16-19, 2009
Meighan Daly, Sabina Sheppard, Michael Huelsmeyer, Heather Wilson

INTRODUCTION: Masitinib mesylate is an oral receptor tyrosine kinase inhibitor (RTKI) developed for the veterinary market with activity against both mutated and wild type forms of the c-kit receptor (CD117), platelet derived growth factor receptors α and β (PDGFR), as well as various other receptors. Both fibroblastic growth factor receptors and PDGFRs are often altered in vaccine associated sarcomas in the cat. The purpose of this study was to evaluate growth inhibition, radiosensitization, and chemosensitization of two feline sarcoma cell lines exposed to masitinib mesylate and to establish the safety of clinically relevant doses in healthy cats.

METHODS: Two feline sarcoma cell lines were evaluated in this study. Reverse transcriptase polymerase chain reaction (RT-PCR) evaluation for CD117 and PDGF receptor was performed. Cells were incubated with varying concentrations of masitinib and evaluated. Masitinib treated and untreated cells were exposed to 5 Gy Cobalt 60 radiation. Treated and untreated cell lines were exposed to doxorubicin and carboplatin. Cell survival was determined using flow cytometry. In healthy cats, 3 cats were started at a dose of 50 mg masitinib (12.5 mg/kg) every other day. A CBC, Chemistry, UA, UPC and blood pressure were collected from each cat at various time points. Daily physical examinations were performed.

RESULTS: Both cell lines were shown to express CD117 and PDGF receptors. Growth inhibition was seen due to masitinib treatment under the various conditions in the cell lines.

CONCLUSIONS: Masitinib has potential as an anti neoplastic agent in the cat for use against vaccine associated sarcomas as well several other diseases.
9 MISCELLANEOUS CANINE AND FELINE TREATMENTS

9.1 MASITINIB – A TARGETED THERAPY WITH APPLICATIONS IN VETERINARY ONCOLOGY AND INFLAMMATORY DISEASES

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com). A pre-publication author version is also available to download from the following weblink: Click to download [https://docs.google.com/viewer?a=v&pid=explorer&chrome=true&srcid=0B4Of9Adqv406YzA5Y2FmNWEtYjU1Ny00MGVlLThhZjQtNDQ2MjYiNDkzNTI4&hl=en_US]

GK Ogilvie, P Hensel, BE Kitchell, P Dubreuil, and A Ahn

SUMMARY
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 signaling 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 tumor (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. Of these, the latter two 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 program 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.
MASITINIB – TARGETED THERAPY IN VETERINARY MEDICINE

Publication Category: Conference Presentation

Albert Ahn

SUMMARY
Tyrosine kinase inhibitors (TKI) are an innovative class of drug that has recently become available to veterinary medicine. This targeted therapy inhibits enzymes involved in cellular signaling pathways that regulate key cell functions and survival. Over the last decade TKIs have revolutionized the management of certain human cancers and are now making inroads into veterinary medicine, with canine mast cell tumors (MCT) being their first major success story. Two TKIs have been specifically developed for the veterinary setting, masitinib and toceranib. It will be masitinib however, with its two completed phase III studies in canine oncology and immune-mediated indications, its long-term follow-up of MCT treatment (approximately 6 years), its wealth of knowledge accumulated from over 2 years of on- and off-label use in Europe, and development program 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.

The first oncological indication approved for masitinib was canine MCT, the most common cutaneous malignant neoplasm in dogs. Masitinib (Kinavet CA-1®) has recently become available to US veterinarians following conditional FDA approval for treatment of recurrent (post-surgery) or non-resectable grade II/III cutaneous MCTs in dogs that have not previously received radiotherapy and/or chemotherapy except corticosteroids. In fact, masitinib was the first ever approved drug for veterinary oncology, having been registered by the EMA in 2008 for the treatment MCTs under the trade name Masivet®. Registration was based upon a successful phase III trial and long-term follow-up data that proved masitinib can significantly improve long-term survival and prevent tumor recurrence.

Masitinib’s therapeutic potential can reach beyond the field of chemotherapy. The main cellular targets of masitinib are mast cells, which are capable of releasing large amounts of pro-inflammatory mediators with a consequence of triggering and sustaining an inflammatory response. Masitinib may therefore provide therapeutic benefit in immune-mediated diseases with mast cell involvement, such as atopic dermatitis, asthma, inflammatory bowel disease and arthritis. This capability was exemplified via a phase III study that showed significant reduction in the symptoms of canine atopic dermatitis. A positive response was evident for treatment-naïve dogs, dogs resistant to ciclosporin and/or corticosteroids, and dogs with severe pruritus, the latter two groups representing populations with high unmet medical need.

Owing to its novel mechanism of action, masitinib holds therapeutic potential for both veterinary oncological and immune-mediated conditions. Emerging clinical evidence also indicates masitinib may be of therapeutic benefit for canine T-cell lymphoma, neurofibrosarcoma, and melanoma; for feline MCT and asthma; and as a chemosensitizer for standard chemotherapies.
9.3 NEW THERAPEUTIC OPTIONS IN VETERINARY ONCOLOGY: TYROSINE KINASE INHIBITORS

Publication Category: Conference Presentation (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)

Proceedings of the 2011 American College of Veterinary Internal Medicine Forum, June 15-18, Denver, USA.
Technician Program (T44)
Albert Ahn

SUMMARY
Tyrosine kinase inhibitors (TKI), sometimes referred to as targeted therapies, have revolutionized the management of human cancer since the introduction of imatinib (Gleevec) in 2001. TKIs are now making inroads into veterinary medicine with mast cell tumors (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; of which the latter two have received regulatory registration in dogs with MCT. In many ways masitinib is the forerunner of such veterinary drug development; historically, developmentally, and also in the depth and breadth of clinical knowledge acquired. It will be masitinib therefore, with its completed phase III studies in canine oncology and immune-mediated indications, its long-term follow-up of MCT treatment, and development program for numerous other canine and feline oncology and non-oncological diseases that will serve as an example of what this remarkably versatile class of therapeutic drug promises to accomplish in veterinary medicine.
SUMMARY
Masitinib, a drug being developed for numerous indications in veterinary and human medicine by AB Science (www.ab-science.com), belongs to a therapeutic class known as targeted therapy. These small molecule drugs selectively inhibit specific tyrosine kinases or their signaling pathways. In the last decade several tyrosine kinase inhibitors (TKI) have been developed for the treatment of cancer and other diseases, the most well-known being imatinib (Gleevec, Novartis), which has dramatically improved the treatment for patients with chronic myelogenous leukemia and gastrointestinal stromal tumor. Unfortunately, resistance to imatinib tends to develop for some patients. Nonetheless, targeted therapies are still creating a buzz in human and veterinary medicine alike; including, at this year’s VCS conference in Las Vegas.

Masitinib is arguably the most specific inhibitor of KIT available. In vitro, it has shown greater affinity and selectivity for the KIT receptor as compared to imatinib, while not inhibiting, at therapeutic doses, those tyrosine kinases or tyrosine kinase receptors attributed to possible toxicity. This highly selective nature suggests that it will exhibit a better safety profile than other TKIs (Dubreuil et al, 2009). Among masitinib’s other claims-to-fame is that it was the first approved targeted therapy in veterinary oncology, having been registered by the European Medicine Agency in November 2008 for the treatment of non-resectable mast cell tumors (MCTs) grade II/III (EMEA approval under the trade name Masivet®; Kinavet® in the USA, pending FDA registration review).

Since Masivet’s European launch in 2009 it is estimated that 480 veterinarians have used it for approximately 1390 dogs; primarily as a treatment for MCT but also as an off-label treatment for various other cancers and in a phase III study for canine atopic dermatitis. In the USA, masitinib has been available as part of a compassionate-use program with approximately 650 recipients to date.
9.5 TARGETED THERAPY WITH MASITINIB IN CANINE AND FELINE TUMOURS IN TWO EUROPEAN VETERINARY ONCOLOGY CENTRES.

Publication Category: Conference Presentation (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)

Johan de Vos and Malcolm Brearley

SUMMARY
Mast cell tumours are the most common malignant skin neoplasm of dogs. Their clinical behavior closely parallels their histopathological characteristics. The vast majority of Grade 1 MCTs are curable by relatively simple surgical excision; whereas the majority of poorly differentiated Grade 3 tumours require more extensive local therapy and have a high probability of metastatic spread. The majority of Grade 2 tumours do not metastasise and are theoretically curable by local therapy. However size and anatomical constraints may prevent this. Therefore although many MCTs are curable by surgery alone or in combination with radiation, there is a significant minority that pose a severe challenge to conventional treatment either because of extensive local disease or metastatic spread.

Recent studies indicated that gain-of-function mutations in the proto-oncogene c-kit are associated with canine mast cell tumour (MCT) development, correlating with a higher histologic grade and opening possibilities for therapeutic strategies. C-Kit, a trans-membrane tyrosine kinase, and its ligand Stem Cell Factor, have important signalling roles in normal mast cell development and function, erythropoiesis, lymphopoiesis, megakaryopoiesis, gametogenesis, and melanogenesis. Gain-of-function mutations cause a constitutive activation of the c-Kit receptor. So far it is not clear whether these mutations are causative for tumour development, or are only complicating the carcinogenesis. C-kit mutations are found in about 30% of canine MCT (predominantly high grade 2 and grade 3), without breed predilection. The majority of the mutations detected in canine MCT are internal tandem duplications, insertions and deletions in exon 11, the coding region of the JM domain. Mutations in exons 8 and 9, which encode for the fifth immunoglobulin-like domain, are less common.

Masitinib specifically targets the tyrosine kinase receptors c-Kit (wild type isoform as well as exon 8, 9 and 11 mutants), platelet-derived growth factor receptors (PDGFR α and β), and the intracellular kinases Lyn, Fyn and Lck. Masitinib competitively binds to the ATP-binding site of the intracellular kinase domain of the receptor, inhibiting phosphorylation and so activation of the receptor. The half-life of masitinib in dogs is 3-6 hours, and the recommended therapeutic canine dose is 12.5 mg/kg s.i.d. orally.

In De Ottenhorst, Veterinary Oncology Referral Centre, The Netherlands and The Queen’s Veterinary School Hospital, University of Cambridge, UK to date 38 tumour bearing dogs and 4 tumour bearing cats were treated with masitinib in the period December 2008 till December 2009. The main tumour type treated was MCT, but also dogs and cats with other tumour types were included.
9.6 DRUG-INDUCED MINIMAL CHANGE NEPHROPATHY IN A DOG

Publication Category: Peer Reviewed Journal (Full manuscript available in appendices or upon request to AB Science - contact@ab-science.com)


SUMMARY
This is a case report from the College of Veterinary Medicine, The University of Georgia. This report describes the clinical signs, laboratory and histopathologic abnormalities, and clinical course of drug-induced minimal change nephropathy in a dog.

9.7 CLINICAL TRIAL ON THE EFFICACY OF MASITINIB IN CANINE IBD

Publication Category: Peer Reviewed Journal

Procoli F.
Vet Rec. 2010 Nov 6;167(19):760. doi: 10.1136/vr.c6030

SUMMARY
IDIOPATHIC inflammatory bowel disease (IBD) is the most common cause of chronic intestinal disease in dogs. Current treatment protocols often involve the use of immunosuppressive doses of corticosteroids to reduce intestinal inflammation and achieve remission. However, a number of dogs with IBD treated with corticosteroids have either no response at all to the drug or relapse after weeks to months of treatment. In addition, treatment with prednisolone often results in unacceptable side effects. Masitinib is a protein tyrosine kinase inhibitor licensed for use in dogs with mast cell tumours, in which it has proven efficacy. There are reports that this drug can decrease inflammation in people with several immune-mediated disorders, including IBD. The Clinical Investigation Centre at the Royal Veterinary College is performing a clinical trial to evaluate the clinical efficacy of masitinib as sole treatment for canine IBD in steroid refractory cases over a period of eight weeks, and would welcome suitable cases. Requirements for enrolment in the trial include exclusion of other causes of chronic diarrhoea (through routine diagnostic tests), the presence of inflammatory cell infiltrates in mucosal biopsies obtained by gastroduodenoscopy and/or colonoscopy (for the diagnosis of IBD) and previous treatment with corticosteroids for at least four weeks with only minimal or no response. Consultation, physical examination, blood work and the costs of the drug will be covered. Please note that the drug will not be dispensed beyond the duration of the study.
10 LIST OF APPENDICES

Full copies of the following manuscripts are available as part of the appendices included in this document, or upon request to AB Science, or from the relevant publication source.

- Masitinib (AB1010), a potent and selective tyrosine kinase inhibitor targeting kit
- Masitinib as a chemosensitizer of canine tumor cell lines: a proof of concept study
- Gain-of-function mutations in the extracellular domain of KIT are common in canine mast cell tumors
- Evaluation of 12- and 24-month survival rates after treatment with masitinib in dogs with nonresectable mast cell tumors
- Masitinib is safe and effective for the treatment of canine mast cell tumors
- Masitinib for the treatment of canine atopic dermatitis: a pilot study
- Safety of masitinib mesylate in healthy cats
- Pharmacokinetics of masitinib in cats
- Masitinib – the efficacy of targeted therapy in veterinary medicine
- Drug-induced minimal change nephropathy in a dog
- Targeted therapy with masitinib in canine and feline tumours in two European veterinary oncology centres
- Masitinib decreases symptoms of canine atopic dermatitis: a multicenter, randomized, double-blind, placebo-controlled phase 3 trial
- Masitinib – a targeted therapy with applications in veterinary oncology and inflammatory diseases
- New therapeutic options in veterinary oncology: tyrosine kinase inhibitors
- Masitinib in canine mast cell disease
- Masitinib demonstrates anti-proliferative and pro-apoptotic activity in primary and metastatic feline injection-site sarcoma cells