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Authors: S. Bavcar, J. de Vos, M. Kessler, P. de Fornel, P. Buracco, S. Murphy, J. Hirschberger, D.J. Argyle

PII: S1090-0233(17)30088-6
DOI: http://dx.doi.org/doi:10.1016/j.tvjl.2017.04.010
Reference: YTVJL 4984

To appear in:

Accepted date: 18-4-2017

Please cite this article as: S.Bavcar, J.de Vos, M.Kessler, P.de Fornel, P.Buracco, S.Murphy, J.Hirschberger, D.J.Argyle, Combination toceranib and lomustine shows frequent high grade toxicities when used for treatment of non-resectable or recurrent mast cell tumours in dogs: A European multicentre study (2010), http://dx.doi.org/10.1016/j.tvjl.2017.04.010

Original Article

Combination toceranib and lomustine shows frequent high grade toxicities when used for treatment of non-resectable or recurrent mast cell tumours in dogs: A European multicentre study

S. Bavcar [a,*], J. de Vos [b], M. Kessler [c], P. de Fornel [d], P. Buracco [e], S. Murphy [f], J. Hirschberger [g], D.J. Argyle [a]

[a] *Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Edinburgh EH25 9RE, United Kingdom*

[b] *De Ottenhorst, Veterinary Oncology Referral Centre, Animal Hospital Zeeuws-Vlaanderen, Terneuzen, The Netherlands*

[c] *Tierklinik Hofheim, Hofheim, Germany*

[d] *Centre Micen Vet, Créteil, France*

[e] *University of Turin, Dipartimento di Scienze Veterinarie, Grugliasco, Torino, Italy*

[f] *Animal Health Trust, Suffolk, United Kingdom*

[g] *Clinic of Small Animal Medicine, Ludwig-Maximilians-Universität München, Germany*

* Corresponding author: Tel.: +44 754 9165040.
E-mail address: spela.bavcar@ed.ac.uk (S. Bavcar).

Highlights

- Non-resectable, recurrent and/or metastatic mast cell tumours (MCTs) in dogs represent a clinical challenge.
- Ten dogs with MCTs were treated with a combination of toceranib, lomustine, and prednisolone.
- The number of significant adverse events was relatively high.
- Neutropenia was the most commonly noted adverse event, but was of low clinical importance.
- Significant gastrointestinal side effects resulted in exclusion of four dogs from the study.

Abstract

Mast cell tumours (MCTs) in dogs can present in a variety of forms. Non-resectable, recurrent or metastatic MCTs usually carry a poor prognosis and present a therapeutic challenge. Both toceranib and lomustine have shown single agent activity against MCTs in dogs. In this study, 10 dogs with advanced MCTs were enrolled prospectively and treated with toceranib (median dose 2.7 mg/kg orally every other day), lomustine (median dose 60 mg/m^2 orally every 3 weeks) and prednisolone (1 mg/kg orally every other day, alternating with toceranib). Severe adverse events (SAEs), requiring alterations in the protocol, occurred in all dogs. The objective response rate was 50%. Three dogs died or were euthanased due to SAEs and therefore enrolment of new dogs was discontinued prematurely. A long term response (> 1 year) was observed in two dogs. Modifications of the protocol are required for future prospective studies.

Keywords: Canine; Mast cell tumour; Toceranib; Lomustine; Adverse events

Introduction

Mast cell tumours (MCTs) are the most common type of cutaneous tumours in dogs and comprise 11-21% of all skin tumours (Bostock, 1986; Villamil et al., 2011). The treatment of choice is wide surgical resection; adjuvant therapy, such as radiation therapy, chemotherapy and receptor tyrosine kinase inhibitors (RTKI), are recommended for improved tumour control in those cases that are not treatable by surgery. Non-resectable, recurrent and/or metastatic MCTs in dogs represent a clinical challenge and often become refractory to treatment with systemic drugs, such as vinblastine, lomustine, prednisolone and RTKI (Rassnick et al., 1999; Thamm et al., 1999, 2006; London et al., 2009; Blackwood et al., 2012; Smrkovski et al., 2013).

Toceranib and lomustine are widely used as single agents in the treatment of MCTs in dogs. Toceranib is a RTKI with activity against a wide range of split receptor tyrosine kinases. These include vascular endothelial growth factor receptor 2 (VEGFR-2), platelet-derived growth factor receptor (PDGFR)-α/ß, stem cell factor receptor c-Kit, fms-related tyrosine kinase 3 (Flt-3) and colony stimulating factor receptor 1 (CSFR1) (Liao et al., 2002; London et al., 2003). Toceranib is approved by the European Medicine Agency (EMA) and the USA Food and Drug Administration (FDA) for the treatment of non-resectable, Patnaik grade II or III (Patnaik et al, 1983), recurrent, cutaneous mast cell tumours in dogs. The most commonly observed adverse events (AEs) related to treatment with toceranib are neutropenia and gastrointestinal (GI) signs (e.g. anorexia, diarrhoea and vomiting), with grade III or IV GI AEs observed in 2.3-9.2% of cases (London et al., 2003). Other AEs, such as proteinuria, lethargy and muscle pain, have also been described (London et al., 2003a and b; Gore et al., 2009). The objective response rate (ORR) in toceranib-treated dogs with recurrent (either local or distant) MCTs following surgical excision was 37.2% in one study (London et al., 2009).

Lomustine, also known as 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), is a cell cycle non-specific DNA alkylating agent used for the treatment of a variety of neoplastic conditions in veterinary medicine (Moore et al., 1999; Kristal et al., 2004; Jung et al., 2006; Risbon et al., 2006; Williams et al., 2006; Saba et al., 2007; Cooper et al., 2009; Rassnick et al., 2010a and b). The most commonly observed AEs associated with lomustine include bone marrow suppression and hepatotoxicity, with GI side effects reported rarely and mainly in association with sepsis (Moore et al., 1999; Rassnick et al., 1999; Kristal et al., 2004). Response rates to treatment with single agent lomustine in dogs with MCTs are 1-42% (Rassnick et al. 1999; Vail at al. 2012), while the reported response rate to treatment with oral prednisolone alone is 20% (McCaw et al., 1994).

The aim of this prospective study was to assess the ORR, progression-free interval (PFI) and toxicity profile of a combined lomustine-toceranib-prednisolone protocol in dogs with high-grade (Patnaik grade II/III) non-resectable and/or recurrent, or metastatic, MCTs. The hypothesis was that a combination protocol with lomustine, toceranib and prednisolone would be well tolerated, since these are drugs with known single agent activity that lack overlapping dose limiting toxicities. It was hypothesised that the protocol would induce responses in > 20% of non-resectable and/or recurrent high grade, or metastatic, MCTs in dogs.

Materials and methods

This prospective European multicentre study was a collaboration of seven universities and veterinary oncology centres across Europe (University of Edinburgh, Hospital for Small Animals, United Kingdom; Veterinary Oncology Referral Centre, Animal Hospital Zeeuws-Vlaanderen, Terneuzen, The Netherlands; Tierklinik Hofheim, Hofheim, Germany; Centre Micen Vet, Créteil, France; University of Turin, Dipartimento di Scienze Veterinarie, Grugliasco, Torino, Italy; Animal Health Trust, Suffolk, United Kingdom, and the Clinic of

Small Animal Medicine, Ludwig-Maximilians-Universität, Munich, Germany). Dogs were enrolled in the study from October 2012 to January 2013.

Selection of subjects

Client owned dogs with measurable primary or recurrent, non-resectable and/or metastatic Patnaik grade II/III MCTs diagnosed on histopathology, with a minimal estimated life expectancy of 8 weeks without treatment, were eligible to be included the study. A written informed consent form was signed by the owners prior to enrolment. Routine MCT staging was performed, including clinical examination, regional lymph node cytology, abdominal ultrasound with or without liver and spleen cytology, thoracic radiography, haematology, serum biochemistry and urinalysis. Evaluation of lungs, liver, spleen and lymph nodes using computed tomography (CT) was also allowed. Pre-treatment with other chemotherapeutic agents within 30 days prior to enrolment or curative intent local treatment was not allowed. Treatment with antihistamines and GI protectants was allowed, but not routinely recorded.

Treatment protocol

The planned doses were 70 mg/m^2 lomustine phosphate orally every 3 weeks and 2.7 mg/kg toceranib (Palladia, Zoetis) orally every other day, starting a day after lomustine, for at least six cycles, unless progressive disease or severe adverse events (SAEs) were reported, with the dose adjusted to the nearest dose that could be administered using available whole capsules of lomustine or tablets of toceranib. The planned dose of prednisolone was 1 mg/kg, administered orally on days alternating with toceranib. Total treatment time was planned to be 6 months or until detection of progressive disease. All target lesions were routinely measured using calipers and digitally photographed on the day of administration of lomustine.

Haematology was performed immediately prior to each dose of lomustine and 1 week after administration of lomustine. Serum biochemistry was repeated every 3 weeks also immediately prior to the planned administration of lomustine. The dose of lomustine was delayed if the neutrophil count was < 2.0 x 10^9/L or the platelet count was < 100 x 10^9/L; in these cases, a 10-15% dose reduction was applied at subsequent administration. When SAEs were encountered, toceranib was withdrawn for a minimum of 1 week when indicated on the judgement of the veterinary clinicians, with a 10-15 % dose reduction when re-started. Dogs were taken out of the study if the platelet count was < 100 x 10^9/L on two or more subsequent analyses, if alanine aminotransferase (ALT) was more than twice the upper reference value of the oncology centre and could not be explained by prednisolone administration (with concurrent elevations of heat stable alkaline phosphatase, ALP), or when other SAEs were refractory to treatment or persisted despite a dose reduction of the medicines included in the protocol.

Assessment of tumour response and toxicity

Response to treatment and treatment related toxicity were monitored according to cRECIST 1.0 (Nguyen et al., 2015) and VCOG-CTCAE v1.1 (VCOG, 2011), respectively, from the time of enrolment of the first dog until the death of the last dog. AEs were considered to be severe when graded III or higher. Complete response (CR) was defined as disappearance of all target lesions. Partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameters of target lesions compared to the base line measurements. Progressive disease (PD) was defined as either the appearance of one or more new lesions, or at least a 20% increase in the sum of the longest diameters of target lesions, relating to the smallest lesion diameter measured during treatment. Stable disease (SD) was defined as neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD. Treatment failure was defined as progressive disease. ORR was defined as the number of

cases that achieved either CR or PR. Progression free interval (PFI) was determined from the date of start of therapy to the date of tumour progression, or tumour or therapy related death. The owner and/or veterinary oncologist were allowed to remove the dog from the study at any time.

Results

Demographics

Ten dogs for which complete data were available were included in the study (Table 1). Breeds included were two Shar Peis and one of each of Jack Russell terrier, Whippet, Labrador, Norwegian Buhund, Hovawart, Golden retriever, Rhodesian ridgeback and cross-breed. Four were neutered male dogs, five were neutered female dogs and one was an entire male dog. The median and mean age was 10 years (range 1 to 12 years). The median weight was 19.0 kg (range 8.0 to 42.3 kg). Six dogs had a single MCT and four had multiple MCTs, of which one dog had multiple metastases in the skin, subcutaneous tissues, and regional and distant lymph nodes.

Treatment details

Individual dogs received one to six doses of lomustine (median two doses), with a dose range of 50.0-88.9 mg/m^2 (median 60 mg/m^2). The dose of toceranib was 2.5-2.8 mg/kg (median 2.7 mg/kg) administered orally every other day. The duration of toceranib treatment was 5-23 weeks (median 11.5 weeks).

Toxicities and side effects

Dose reductions and/or treatment delays were necessary in all 10 dogs due to dose limiting toxicities (DLTs). The total number of DLTs was 26 (median two per dog; range one to six per dog). Grade 3 and 4 neutropenia, requiring dose delay and dose reduction, occurred 15 times in nine dogs (median one episode per dog; range one to four episodes per dog). Treatment with prophylactic antibiotics was administered on six occasions when grade III or

grade IV neutropenia was documented. Two dogs developed pyrexia, one with grade III and one with grade IV neutropenia, which resolved during intravenous fluid therapy and antibiotic treatment.

Severe GI toxicity was documented nine times in five dogs, and presented as nausea/anorexia (n = 1), vomiting (n = 6) and diarrhoea (n = 2). One dog developed severe pancreatitis and another developed grade IV hepatotoxicity, with ALT exceeding 10 times the upper reference value of the centre following administration of five doses of lomustine.

Toceranib had to be temporarily discontinued in nine dogs during the treatment protocol. The number of necessary drug withdrawals in these nine dogs ranged from one to four (median two) per dog, lasting on average for 1 week (range 1-2 weeks).

Three dogs were removed from the study due to protocol intolerance at 35 days (n = 1) and 56 days (n = 2) after starting treatment, two due to a combination of GI SAEs and neutropenia, and one due to recurrent severe neutropenia despite multiple dose reductions. One dog with PR was lost to follow up after the last recheck at 385 days. Six dogs died or were euthanased, two due to PD, three due to treatment-related side effects that included severe pancreatitis (n = 1) and severe persistent GI toxicity (n = 2), and one dog due to renal failure while in CR.

Response to treatment

Of the 10 dogs in which response could be assessed, three dogs had CR, two had PR, three had SD and two had PD, providing an ORR of 50%. Of the dogs that showed an objective response, PFI ranged from 35 to 613 days, with a median of 86 days (Fig. 1). The longest surviving dog was diagnosed with a grade III MCT, and multiple regional and distant metastasis, and died of renal failure while assumed to be in CR, 613 days after starting treatment.

Discussion

Combining several anti-tumour medications for the treatment of canine MCTs has been shown to increase response rates (Camps-Palau et al., 2007; Cooper et al., 2009; Rassnick et al., 2010). However, drug-drug interactions may occur when a patient's response to the drug is modified by co-exposure to another drug. Factors affecting drug metabolism in general are age and sex, genetic polymorphisms leading to variations in pharmacogenetics/pharmacogenomics, biotransformational polymorphisms leading to inability to remove drugs, genetic polymorphisms in cytochrome P450 (CYP), diet and organ dysfunction. Hypothetically, single nucleotide polymorphisms in CYP sub-enzymes can influence the rate of metabolism of RTKI and other co-administered drugs.

Differences in drug-drug interactions outcomes are generally negligible because of the wide therapeutic window of common drugs. However, for anticancer agents, serious clinical consequences may occur from small changes in drug metabolism and pharmacokinetics (van Leeuwen et al., 2014). By understanding the pharmacokinetic profile of these drugs and their similarities, factors that influence drug exposure will be better recognised and this knowledge may be used to limit sub-therapeutic or supra-therapeutic drug exposure.

In human beings, the main CYP enzyme, CYP3A4, is implicated in the metabolism of most administered drugs, as well as the majority of the RTKIs, with other CYP-enzymes playing a secondary role. Therefore, there is potential for interaction between RTKI and other drugs that modulate the activity of this metabolic pathway. Cancer patients are susceptible to drug-drug interactions, since they often receive many medications. Various clinically relevant drug interactions with RTKI have been identified in human oncology (Erp van et al., 2009). However, sparse literature is available on the metabolism of lomustine and toceranib in dogs, mainly focussing on metabolite formation (Yancey et al., 2010; Thushara et al., 2014), and no clear data are available to indicate the exact pathways of metabolism of toceranib and lomustine in dogs. When the current protocol was designed, overlapping toxicity between the two drugs

was considered to be low and expected to be mainly limited to bone marrow suppression; however, the number of SAEs turned out to be relatively high.

The most frequently encountered DLT was neutropenia (n = 15), resulting in a relatively high number of dose delays, dose reductions and drug holidays. This has also been described in a phase I study, where myelosuppression, specifically neutropenia, was higher compared with each drug's known AE profile when used alone in non-mast cell tumour bearing dogs treated with a combination of toceranib and lomustine (Pan et al., 2016), as well as during treatment of unresectable MCTs with pulse-administration of same two drugs (Burton et al., 2015). The main difference between these two studies is the dose intensity of administered toceranib. In the study of Pan et al. (2016), toceranib was administered every other day at standard doses (2.75 mg/kg), versus pulse administration of toceranib in the study of Burton et al. (2015) (2.75 mg/kg on days 1, 3 and 5 of a 21 day cycle). The continuous administration of toceranib in the current study, more closely resembling the study of Pan et al. (2016), might have contributed to the high incidence of neutropenia; however, this dosing scheme was considered to be appropriate, since it more closely resembles the labelled dose for toceranib (London et al., 2003). Neutropenia was the DLT in two other phase I studies when combining toceranib with other cytotoxic agents, such as doxorubicin and vinblastine (Robat et al., 2012; Pelin et al., 2016). Neutropenia has also been identified in dogs with a range of cancers, including MCTs, treated with lomustine in a subsequent study by Burton et al. (2016). Despite the high number of cases with grade III and IV neutropenia in current study, this was not a cause of death in any of the three cases that were considered to have died due to treatment related complications; however, neutropenia was a cause for withdrawal of one dog from the study out of 13 initially recruited.

Hepatotoxicity was reported in only one dog in our study; this was unexpected given the results of the study by Burton et al. (2015), using combined therapy with lomustine (47.6

mg/m^2) and toceranib in dogs with MCTs, in which 59% of dogs had elevated ALT, even though our study used a higher average single dose of lomustine (60 mg/m^2). No prophylactic treatment for hepatotoxicity was administered in the current study. Lomustine-related hepatic injury appears to be localised to the large bile ducts and includes oedema, bile stasis and degeneration of epithelial cells, leading to pericholangitis, intrahepatic cholestasis, secondary hepatocyte injury, mild bile duct hyperplasia, fibrosis and, eventually, biliary cirrhosis (Kretschmer et al., 1987; Kristal et al., 2004). We hypothesise that, since all dogs in the current study were on an immunosuppressive dose of prednisolone, this could have prevented lomustine related hepatotoxicity. However, with a median of only two doses, most dogs did not receive a high cumulative dose of lomustine and the follow up time was short, so the incidence of hepatotoxicity using our protocol cannot be fully assessed. The serum biochemistry panel used for monitoring of dogs during treatment was limited to assessment of AP and ALT levels, whereas other liver parameters, such as bile acids and bilirubin, were not routinely assessed. Assessment of additional parameters indicating hepatic injury might have revealed a higher incidence of hepatotoxicity.

Significant GI side effects were reported nine times in five dogs. This is less frequent than in other studies (Burton et al., 2015; Pan et al., 2016), but the clinical signs were less responsive to symptomatic management, resulting in two dogs being removed from the study prematurely and owners opting for euthanasia in two dogs with refractory GI toxicity. Owner/clinician bias cannot be excluded in these cases, since these owners might have been less tolerant towards the GI toxicity in their pets, and therefore may have opted for exclusion from the study or euthanasia in cases that failed to promptly recover with symptomatic management. Another explanation for significant GI effects might be the effects of degranulation due to the MCT. Since the use of antihistamines and GI protectants was not routinely recorded, this cannot be completely discarded; however, there were no other clinical

signs, such as erythema and local or systemic coagulation abnormalities, related to the release of vasoactive substances. Severe pancreatitis, unresponsive to supportive care, was the cause of death in one dog. Pancreatitis has not been recognised as an adverse event in treatment with lomustine, but has been reported in a few dogs receiving toceranib (Bernable et al., 2013). There were no observable differences in tumour burden, drug doses or body weight of these dogs. Post-mortem investigations were not performed in these dogs and, therefore, infiltrative MCT disease could not be excluded as a cause of the clinical signs; however, there were no abnormal imaging findings on initial staging.

A wide range of doses of lomustine was administered to dogs in the present study (50.0-88.9 mg/m^2; median 60 mg/m^2). Lomustine is routinely available in 40 mg capsules and compounded forms are not readily available in practice. However, none of the administered doses exceeded the maximum tolerated dose (MTD) of 90 mg/m^2 in dogs (Moore et al., 1999; Kristal et al., 2004). Similarly, due to the available tablets of 10, 15 and 50 mg, doses of toceranib ranged from 2.5 to 2.8 mg/kg orally every other day, so the labelled dose of 3.25 mg/kg orally every other day (London et al., 2003) was not exceeded.

The ORR of 50% in the current study was considerably higher than recently reported for lomustine in a randomised, double blind, positive controlled confirmatory clinical field study evaluating the efficacy and safety of water soluble micellar paclitaxel (Vail et al., 2012), where the confirmed overall response rate and the biological observed response rate of lomustine were reported to be 1% and 10%, respectively, but was comparable to other single agent protocols (Rassnick et al., 1999; Thamm et al., 1999; London et al., 2009; Smrkovski et al., 2013). However, dogs in the current study did not receive surgery, which was included in patient management in other studies (Thamm et al., 1999; London et al., 2009a).

The main limitation of the current study is the low number of cases (n = 10) due to a high frequency of side effects (SAEs) reported in the early phase of data collection, resulting

in premature discontinuation of new subject enrolment; as a consequence, direct comparison of results with previous studies is not possible. The MTD of lomustine given with continuous toceranib has been determined by Pan et al. (2016) to be 50 mg/m^2 administered every 3 weeks. Other limitations of the present study include the multi-centre study design and lack of a control group, making comparison of toxicities and responses to treatment challenging.

Conclusions

Whilst the ORR of 50% was promising, the combination of toceranib, lomustine and prednisolone was associated with significant toxicity, since all dogs required some modification to the protocol, and 30% died or were euthanased due to treatment-related toxicity. The number of dogs included in the study was small because due to the frequency and severity of side effects, which resulted in premature termination of the trial. There is a need for a better understanding of the pathways of metabolism of toceranib and lomustine. Alteration of drug dosages and/or frequency will be needed to improve tolerability, while optimising the response of canine MCTs to therapy.

Conflict of interest statement

None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

The authors wish to express, with regret, the sad passing of Dr Johan de Vos, the second author on this paper, who made a major contribution to this study. He will be missed by all of his friends and colleagues. The authors also wish to thank all owners and their dogs for participating in the study, Zoetis for providing financial support, and enabling recruitment and treatment of the dogs, and Ian Handel for his help in analysing the data.

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Table 1

Treatment details of dogs included in the trial.

	Category	*n*
Lomustine	Number of doses (range)	29 (1-6)
	Dose in mg/m^2 (range)	60 (50-88.9)
Toceranib	Dose in mg/kg (range)	2.70 (2.50-2.79)
	Duration (weeks)	11.5 (5-23)
DLTs	Total number	26
	Median/dog (range)	2 (1-6)
Neutropenia (Grade III and IV)	Total number	15
	Median/dog (range)	1 (1-4)
Prophylactic antibiotics	Total number	6
Pyrexia	Total number	2
GI toxicity (grade III or IV)	Total number	9
	Nausea	1
	Vomiting	6
	Diarrhoea	2
Other toxicities	Pancreatitis (grade IV)	1
	Hepatotoxicity (grade IV)	1
Drug withdrawal periods (toceranib)	Total number	9
	Median/dog (range)	2 (1-4)
	Median duration in weeks (range)	1 (1-2)
Response	Complete remission (CR)	3
	Partial remission (PR)	2

Stable disease (SD)	3
Progressive disease (PD)	2
Overall response rate (OOR)	50%

MCT, mast cell tumour; DLT, dose limiting toxicity; GI, gastrointestinal.

Figure legend

Fig. 1. Kaplan-Meier curve presenting progression free survival (PFI) in 10 dogs. The first vertical bar represents a dog that was lost to follow up (at 385 days); the second vertical bar represents a dog that died in complete remission (CR) at 613 days.

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