PAPER

Development and progression of proteinuria in dogs treated with masitinib for neoplasia: 28 cases (2010-2019)

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Objectives: To describe the incidence, severity and progression of proteinuria over the first 6 months of masitinib treatment in tumour-bearing dogs without pre-existing proteinuria. To describe the effect of treatment on urine protein:creatinine and renal parameters in patients with pre-existing proteinuria.

Materials and Methods: Records were reviewed from patients receiving masitinib for neoplasms between June 1, 2010, and May 5, 2019. Patients without pre-treatment and at least one urine protein:creatinine after ≥7 days treatment were excluded. Signalment, tumours and concurrent diseases, treatments, haematology, biochemistry and urinalysis results before, during and after treatment for up to 202 days were collected. Patient visits were grouped into six timepoints for analysis.

Results: Twenty-eight dogs were included. Eighteen percent of dogs non-proteinuric at baseline (four of 22) developed proteinuria during treatment, all within 1 month of treatment initiation. One dog developed hypoalbuminaemia, none developed oedema or ascites, azotaemia or were euthanased/died due to proteinuria. Masitinib was immediately discontinued in both dogs in which urine protein:creatinine greater than 2.0 was detected and in both, proteinuria improved.

Six dogs with pre-treatment proteinuria were treated with masitinib, significant worsening of proteinuria did not occur. Neither azotaemia nor severe hypoalbuminaemia occurred.

Clinical Significance: Proteinuria, when it occurs, tends to develop within 1 month of masitinib commencement and may progress rapidly. Weekly proteinuria monitoring should be considered for the first month and a urine protein:creatinine greater than 0.5 should prompt reassessment within 1 week. Masitinib treatment can be considered in patients with pre-treatment proteinuria and does not inevitably cause worsening of proteinuria.

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INTRODUCTION

Masitinib (Masivet; AB Science) is a tyrosine kinase inhibitor (TKI) licensed to treat canine non-resectable grade II or III mast cell tumours (MCT) with a confirmed mutation of the tyrosine kinase (TK) receptor, KIT. Off-licence use for other MCT, malignant melanoma (MM), epitheliotropic lymphoma (EL) and for atopic dermatitis management has also been reported (Hahn *et al.* 2008, AB Science 2009, Cadot *et al.* 2011, Devine & Polzin 2016, Holtermann *et al.* 2016, Miller *et al.* 2016, Giuliano & Dobson 2020).

Tyrosine kinases selectively phosphorylate other proteins, functioning as important mediators in many cellular signalling

The study was granted ethical approval by the local committee.

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pathways. Masitinib binds to specific TK receptors (including stem cell factor receptor (KIT)) inhibiting their function (Dubreuil *et al.* 2009). As TK receptors are ubiquitous in the body, their inhibition can have undesirable effects on normal tissue causing toxicity. Although normally well tolerated, gastrointestinal, haematological, hepatic and renal adverse events are reported (Hahn *et al.* 2008; Smrkovski *et al.* 2015).

Three case reports have documented development of marked proteinuria and severe hypoalbuminaemia in dogs receiving masitinib (Sum *et al.* 2010, Brown *et al.* 2013, Devine & Polzin 2016). Two also subsequently developed acute kidney injury, one of which was ultimately euthanased. Dogs receiving masitinib have a documented incidence of proteinuria of 16 to 23% but not all develop severe consequences (Cadot *et al.* 2011, Smrkovski *et al.* 2015). The masitinib datasheet stipulates that monthly urine dipstick monitoring should be performed, if proteinuria develops it should be quantified and treatment should be temporarily discontinued if the urine protein to creatinine ratio (UP:C) exceeds 2.0 (AB Science 2009). The studies reporting proteinuria with masitinib give limited information on the severity, timing of development, progression and outcome in affected dogs. A better understanding of the development and course of proteinuria is needed.

Normally, small amounts of protein pass through the renal glomerular filtration barrier (GFB) and are reabsorbed in the proximal tubules, therefore only small amounts of protein are detected in urine. When UP:C exceeds 0.5, significant proteinuria is present (Harley & Langston 2012). Proteinuria may be pre-renal, renal or post-renal. Pre-renal proteinuria occurs when large amounts of low molecular weight plasma proteins are present and are filtered, overwhelming the proximal tubule's resorptive capacity (e.g. haemoglobinuria). Post-renal proteinuria occurs when protein enters urine after the renal pelvis (e.g. cystitis). Renal proteinuria may be either physiological and transient (e.g. fever, seizures or intense exercise) or pathological. Pathological renal proteinuria can be either glomerular or tubular in origin. Glomerular proteinuria implies an alteration in structure or function of the GFB, whilst tubular proteinuria can occur either due to defects in tubular resorptive mechanisms (e.g. Fanconi syndrome) or through a reduction in nephron numbers or altered haemodynamics secondary to tubulointerstitial inflammation. The greater the magnitude of renal proteinuria, the more likely the underlying aetiology is glomerular in origin, particularly if the UP:C is greater than two (Lees *et al.* 2005).

Proteinuria associated with masitinib treatment is believed to be glomerular. In two cases in which renal tissue was collected, negligible glomerular changes were seen on light microscopy, but global effacement of podocyte foot processes was found on electron microscopy, consistent with minimal change disease (Sum *et al.* 2010, Brown *et al.* 2013). The mechanism by which this happens is unclear. In addition to inhibition of KIT, in-vitro inhibition of platelet-derived growth factor receptor (PDGFR) α and β, Lyn kinase and fibroblast growth factor receptor 3 have been reported with masitinib (Dubreuil *et al.* 2009). Although normal glomerular cells do not express KIT, glomerular cells of primates do express PDGFRs (Alpers *et al.* 1993, Miliaras *et al.* 2004). This may also be true in dogs and inhibition of this receptor may result in reduced podocyte integrity in susceptible animals (Sum *et al.* 2010). Alternatively, proteinuria may be mediated through inhibition of another receptor or the development of hypertension. Hypertension is a recognised complication of human TKI use (Chu *et al.* 2007, Azizi *et al.* 2008) and increases in canine systolic blood pressure (SBP) were reported with TKI administration (toceranib phosphate) (Tjostheim *et al.* 2016). However, in two case reports of masitinib-associated proteinuria, hypertension was not documented (Sum *et al.* 2010, Devine & Polzin 2016). Possibly, masitinib's more selective TK inhibition profile (specifically a lack of vascular endothelial growth factor receptor (VEGFR) inhibition) means hypertension does not occur (Dubreuil *et al.* 2009).

The datasheet stipulates that masitinib should not be administered to dogs with a pre-treatment UP:C exceeding 2.0 (AB Science 2009). Its use in dogs with pre-existing proteinuria (UP:C >0.5) has not been reported. Fifteen percent of dogs presented to an oncology service were proteinuric at presentation (Prudic *et al.* 2018) but these patients may not have pathological renal proteinuria or be predisposed to masitinib-associated glomerular pathology. In some dogs, masitinib may be the only remaining treatment choice when other options have been exhausted. Information reporting masitinib's effects in proteinuric dogs is therefore needed.

This study's aims were to (1) describe the incidence, severity and progression of proteinuria over the first 6 months of masitinib treatment in tumour-bearing dogs without pre-existing proteinuria and (2) describe the effect of treatment on UP:C and renal parameters in dogs with pre-existing proteinuria.

MATERIALS AND METHODS

Clinical records of dogs referred to a university teaching hospital were retrospectively evaluated. The database was searched for all dogs charged the item masitinib between June 1, 2010, and May 31, 2019. Dogs were subsequently excluded if they were not diagnosed with neoplasia, did not have a urinalysis (including UP:C) performed within 1 month before starting masitinib, were not treated with masitinib for ≥7 days or did not have follow-up urinalysis (including UP:C) after a minimum of 7 days masitinib treatment.

Patient information collected is shown in Table 1. The study period was defined as the day of masitinib commencement to day 202. Follow-up information, including date and reason for both masitinib discontinuation and for death, was obtained from the records or via phone calls to owners or referring veterinary surgeons.

Urinalysis results were only included if they were performed at a reference laboratory. At each timepoint, the urine was classified as non-proteinuric (UP:C ≤ 0.5) or proteinuric (UP:C >0.5). Proteinuria was then categorised as likely pre-renal, post-renal, physiological renal or pathological renal. Inclusion in several categories was possible. Pre-renal proteinuria was excluded if; total protein was within reference limits and if haematocrit was ≥30%, there was less than '+' blood on urine dipstick and if there was

Table 1. Patient information collected from the records and collection time points

Background data	Signalment, weight, tumour type, grade, date
Baseline data collected at masitinib initiation (denoted T0)	Previous treatment details, presence of gross disease, current clinical signs, concurrent diseases, concurrent medications (including doses) for neoplasm and other comorbidities, date and results of haematology, biochemistry, urinalysis and urine cultures (performed within the 21 days preceding masitinib commencement), method of urine sample collection, laboratory used for urinalysis, date masitinib started, masitinib dose administered
Data collected at: T1 (7 to 20 days from T0)† T2 (21 to 34 days from T0)† T3 (35 to 62 days from T0)† T4 (63 to 90 days from T0)† T5 (91 to 146 days from T0)† T6 (147 to 202 days from T0)†	Appointment date, weight, changes to medication administration or dosing since previous visit (including drug holidays), presence of gross disease, current clinical signs, dates and results of haematology and biochemistry profiles and urinalysis (including UP:C), method of urine sample collection, laboratory used for urinalysis, ongoing medication plans

†If a dog had more than one visit during a time-period, the visit closest to the midpoint of the period with a urinalysis was selected. The study period was defined as the day of masitinib commencement to day 202

no mention of muscle pain or stiffness or creatine kinase was normal. Post-renal proteinuria was excluded if no lower urinary tract signs (stranguria or dysuria), vaginal or preputial discharge or signs of prostatic disease were recorded and if less than five white blood cells per high power field (hpf) or less than "++" bacteria were recorded on urine microscopy and urine culture (if performed) was negative. Physiological renal proteinuria was excluded if there was normothermia and no seizures or intensive exercise within 48 hours preceding urine collection. Pathological renal proteinuria was assumed possible in all proteinuric cases.

For dogs without proteinuria at baseline, proteinuria was also classified using Veterinary cooperative oncology group – common terminology criteria for adverse events (VCOG-CTAE) (VCOG 2016); grade I: UP:C greater than 0.5 but less than 1.0, grade II: UP:Cgreater than or equal to 1.0 for less than 14 days duration and grade III: UP:C greater than or equal to 1.0 for greater than or equal to 14 days.

Statistical analysis

Dogs were grouped based on the presence or absence of proteinuria at baseline. Non-proteinuric dogs were further divided based on whether proteinuria developed on treatment during the study. Findings were described and analysed at baseline and during masitinib treatment for these groups using Microsoft Excel (Washington, USA) and Minitab 18 (Minitab Inc., PA, USA). Continuous data were expressed as medians and ranges. The Kaplan–Meier method with right censoring was used to estimate the median duration of masitinib administration. Further statistical analyses were not performed due to the small data set.

RESULTS

Thirty-eight dogs were identified for inclusion. Ten were subsequently excluded due to less than 7 days treatment (n=1), no pre-treatment UP:C within 1 month (n=5) or no UP:C on masitinib (n=4). Twenty-eight dogs were included.

Patient parameters are shown in Table 2. Five dogs were being treated for EL, one for vulval lymphoma, one for MM and 21 for MCT. Four (19.0%) dogs with MCT had subcutaneous tumours of which three had less than four mitoses per 10 hpf. Mitotic index was unknown in one. For the 17 dogs with cutaneous MCTs, the grades were: Patnaik grade I (n=1), Patnaik grade II (n=4), Patnaik grade III (n=4), Kiupel low grade (n=1), Kiupel high grade (n=6) and unknown (n=1).

Urine was collected a median of 1 day before starting masitinib (range: 0 to 22). Twenty-two (79%) dogs were non-proteinuric (UP:C < 0.5) and six were proteinuric at baseline.

Of the 104 urine samples included, the methods of urine collection were free catch (n=31), cystocentesis (n=2) and unknown (n=71). Ninety-six (92.3%) of urine samples were analysed at the university reference laboratory (see Table 3).

Dogs without pre-treatment proteinuria (dogs 1 to 22)

In this group, three dogs were treated for EL, one for vulval lymphoma, one for MM and 17 for MCT. Of the 17 dogs with MCT, three were receiving prednisolone, four were receiving histamine-1-receptor antagonists, one an histamine-2-receptor antagonist and four were receiving both histamine-receptor antagonists. The dog with vulval lymphoma was receiving antibiotics. The other dogs were not receiving treatment for their tumours. Eight dogs had co-morbidities at baseline and six were on treatment for these (Dog-8, -9, -11, -12, -18 and -19) (Table 4).

Creatinine was within reference limits in all dogs. Two dogs had low albumin (24 and 25 g/L) (reference range (RR): 29 to 36). Table 2 lists other baseline parameters.

Effect of masitinib treatment

Median masitinib starting dose was 11.66 mg/kg (range: 9.66 to 13.64). Two dogs had 4-day drug holidays (for hyporexia (dog-1) and for hyporexia and vomiting (dog-11)). Six dogs had masitinib dose reductions (10 to 50%). These were for neutropenia (five dogs) and thrombocytopenia (one dog).

Median UP:Cs were 0.05, 0.03, 0.07, 0.07, 0.05 and 0.03 at T1 to T6 respectively. Four (18.2%) dogs became proteinuric during the study period (Table 3). None became azotaemic. Table 2 shows parameters for dogs that did and did not develop proteinuria within 202 days of masitinib commencement.

Table 2. Clinical parameters for the dogs that were non-proteinuric at baseline that developed and did not develop proteinuria within the first 202 days of masitinib treatment and those with pre-treatment proteinuria

Parameter	Non-proteinuric prior to masitinib treatment		Proteinuric prior to masitinib treatment (n=6)
	Developed proteinuria (n=4)	Did not develop proteinuria (n=18)	
Sex	1 ME, 1 MN, 1 FE, 1 FN	3 ME, 5 MN, 0 FE, 10 FN	3 ME, 0 MN, 0 FE, 3 FN
Median age in years (range)	9.0 (4.3–11.1)	8.0 (4.5 to 12.2)	10.3 (4.6 to 13.6)
Breeds	Jack Russell terrier (n=1), Dogue de Bordeaux (n=1), crossbreed (n=1), lurcher (n=1).	Labrador retriever (n=4), boxer (n=2), crossbreed (n=2), Jack Russell terrier (n=1), West Highland white terrier (n=1) golden retriever (n=1), French bulldog (n=1), Boston terrier (n=1), beagle (n=1), Doberman pincher (n=1), German pointer (n=1), springer spaniel (n=1), Weimaraner (n=1).	Labrador retriever (n=1), golden retriever (n=1), West Highland white terrier (n=1), Hungarian vizsla (n=1), crossbreed (n=1), bullmastiff (n=1)
Median weight in kg (range)	22.0 (8.0–54.0)	31.7 (6.0–39.0)	29.6 (14.2–64.4)
Tumour type	3 MCT, 1 EL	14 MCT, 2 EL, 1 vulva lymphoma, 1 MM	2 EL, 4 MCT
Number in which gross disease present at baseline	4 (100%)	15 (83%)	6 (100%)
Median number of days between diagnosis and starting masitinib (range)	108 (22 to 668)	56 (18 to 701)	107 (6 to 380)
Number which had received previous chemotherapy	2 (50%)	6 (33%)	5 (83%)
Median time since previous chemotherapy in days (range)	41 and 552 days	56 (12 to 217)	22 (15 to 69)
Number of dogs with co-morbidities	1 (25%)	6 (33%)	2 (33%)
Number of dogs receiving prednisolone at baseline	0	3 (17%)	4 (66%)
Median creatinine (µmol/L) at baseline (range) (reference range: 45 to155)	100 (65 to 134)	86 (66 to 141)	96 (76 to 132)
Number of dogs with low albumin at baseline (reference range: 29 to 36)	1 (25%)	1 (6%)	3 (50%)
Median albumin (mg/L) at baseline (range)	32 (25 to 37)	32 (24 to 38)	29 (23 to 30)
Number of dogs with USG ≥1.030 at baseline	1 (33%)†	11 (61%)	1 (17%)
Median UP:C at baseline (range)	0.05 (0 to 0.45)	0.15 (0.02 to 0.20)	1.68 (0.66 to 4.40)
Median starting masitinib dose in mg/kg (range)	12.33 (11.10 to 13.60)	11.61 (9.70 to 12.50)	11.67 (5.70 to 12.70)‡
Median masitinib dose in mg/kg (range)	11.59 (10.98 to 12.82)	11.17 (6.30 to 12.40)	11.42 (4.21 to 12.71)
Median duration of masitinib treatment in days (range) [number censored§]	31 (18 to 202§) [1]	97 (15 to 202§) [6]	32 (34 to 60) [0]
Number of dogs on masitinib at end of study (day 202)	1 (25%)	6 (33%)	0
Median number of timepoints at which blood and urinalysis were available on masitinib per dog (range)	3 (1 to 5)	3 (1 to 5)	2 (1 to 3)
Median time of last UP:C on masitinib during the study (range)	38 (15 to 179)	82 (9 to 197)	29 (26 to 46)

ME Male entire, MN Male neutered, FE Female entire, FN Female neutered, MCT Mast cell tumour, CEL Cutaneous epitheliotropic lymphoma, MM Malignant melanoma, USG Urine specific gravity, UP:C Urine protein to creatinine ratio
†The urine specific gravity was not available for one dog in this group at baseline.
‡One dog (dog 23) was on less than 50% of the recommended starting dose of 12.5 mg/kg. The next lowest dose was 10.6 mg/kg.
§Data were available beyond 202 days but were right censored at this point if dogs were still on treatment as it was the end of the study

Effect of masitinib treatment – Dogs that developed proteinuria

The maximum UP:C on treatment were 1.06, 1.27, 4.56 and 8.2 (Fig. 1). None of these dogs had masitinib dose reductions or drug holidays. Median time to first detection of proteinuria was 14.5 days (range: 13 to 31). In one dog (dog-6), UP:C was normal on treatment at a previous visit. Proteinuria was categorised as pathological renal in two dogs (dog-6 and dog-21) and either pathological renal and/or post-renal in 2 (dog-4 and dog-8). A urine culture performed 8 days after proteinuria was detected in dog-4 was negative despite no antibiotic administration in the interim, suggesting bacterial infection as a cause of post-renal proteinuria was unlikely. Proteinuria was VCOG-CTCAE grade III in one dog (dog-6) and either grade II or III in three.

Table 3. Presence or absence of proteinuria at each timepoint and outcome in dogs without proteinuria at baseline

Dog Number	T0 (baseline)	T1 (days 7 to 20)	T2 (days 21 to 34)	T3 (days 35 to 62)	T4 (days 63 to 90)	T5 (days 91 to 146)	T6 (days 147 to 202)
1	NP	NP	Dead	Dead	Dead	Dead	Dead
2	NP	NP	Off masitinib	Off masitinib	Off masitinib	Off masitinib	Dead
3	NP	NP	Off masitinib	Off masitinib	Off masitinib	Dead	Dead
4	NP	**P**	Off masitinib	Off masitinib	Dead	Dead	Dead
5	NP	NP	NP	Off masitinib	Dead	Dead	Dead
6	NP	NP†	**P**	Off masitinib	Off masitinib	Off masitinib	Off masitinib
7	NP†	Missing	Missing	NP	Dead	Dead	Dead
8	NP	**P**	NP	NP	Off masitinib	Dead	Dead
9	NP	NP	Missing	Missing	Off masitinib	Dead	Dead
10	NP	NP	NP	NP	Missing	Dead	Dead
11	NP†	NP	NP	NP	NP	Dead	Dead
12	NP	NP	NP	NP	NP	Off masitinib	Off masitinib
13	NP	NP	Missing	NP	Missing	Off masitinib	Off masitinib
14	NP	Missing	NP	NP	Missing	NP†	Off masitinib
15	NP	NP	Missing	NP	Missing	NP	Off masitinib
16	NP	NP	Missing	NP	NP	NP	NP
17	NP	NP	NP	Missing	NP	NP	NP
18	NP	NP	NP	Missing	NP	Missing	NP
19	NP	NP	NP	Missing	NP	NP	Missing
20	NP†	NP†	NP†	NP†	Missing	NP	NP
21	NP	**P**	**P**	**P**	NP	Missing	NP
22	NP	NP	NP	NP	NP	NP	Missing

NP Non-proteinuric (UP:C <0.5), P Proteinuric (UP:C ≥0.5)
T0 to T6 – timepoints
†Samples that did not have UP:C measured at our university reference laboratory.

Table 4. Co-morbidities and associated treatments

	Dog number	Co-morbidities	Medication
Dogs without proteinuria at baseline	5	Atopy	None
	8	Diabetes mellitus	Lente insulin (Caninsulin; MSD Animal Health)
	9	Paw laceration	Amoxicillin and clavulanic acid (Synulox; Zoetis)
	11	Atopy	Oclacitinib (Apoquel; Zoetis)
	12	Arthritis, idiopathic epilepsy	Glucosamines (Senoquin; VetPlus), phenobarbitone (Epiphen; Vetoquinol), firocoxib (Previcox; Boehringer Ingelheim)
	16	Arthritis	None
	18	Mitral valve disease, pulmonary hypertension	Pimobendan (Vetmedin; Boehringer Ingelheim)
	19	Atopy	Dexamethasone ear drops (Maxidex; Alcon), fusidic acid and betamethasone topical cream (Isaderm; Dechra) and hyposensitisation vaccine (Artuvetrin; Artuvet)
Dogs with proteinuria at baseline	23	Renal proteinuria, systemic hypertension (controlled), hypothyroidism (controlled), subaortic stenosis	Benazepril (Fortekor; Elanco), amlodipine (Istin; Pfizer) and levothyroxine (Thyforun; Dechra)
	26	Idiopathic pulmonary fibrosis	None

When dog-4 developed proteinuria (UP:C 8.2) at T1, albumin and USG were unchanged (albumin at T0: 25 g/L; T1: 27 g/L and USG at T0: 1.012; T1: 1.015), but creatinine increased markedly (T0: 75 µmoL/L; T1: 108 µmoL/L). Ten days before T1 (but after T0), oral prednisolone (Prednidale; Dechra) had been started (0.45 mg/kg twice daily) and omeprazole (Omeprazole; Actavis) was stopped. Masitinib was discontinued when proteinuria was documented but prednisolone was continued at the same dose. One week later, the UP:C was 2.53, creatinine and albumin remained stable. Euthanasia occurred 2 weeks later due to disease progression.

In dog-6, albumin markedly declined (T0: 37 g/L; T1: 41 g/L; T2: 21 g/L) when proteinuria (UP:C 4.56) was detected at T2. Creatinine remained stable (T0: 135 µmoL/L; T1: 132 µmoL/L; T2: 135 µmoL/L). Masitinib was discontinued when proteinuria was identified. Four days later, the dog was lethargic, hyporexic and vomiting with evidence of MCT progression. The UP:C was 15.0 and albumin was 13 g/L. Neither oedema nor ascites were documented. Creatinine had increased slightly (150 µmoL/L) but USG was unchanged (T0: >1.050; T1: 1.045; T2: >1.050). Systolic blood pressure was normal. Benazepril (Fortekor; Elanco), omeprazole (Omeprazole; Actavis), famotidine (Famotidine; Tillomed Laboratories), maropitant (Cerenia; Zoetis) and prednisolone (Prednidale; Dechra) (0.57 mg/kg once daily) were started. Eight days after masitinib discontinuation the dog was clinically well, UP:C was 2.92, albumin had increased (19 g/L) and creatinine had reduced (125 µmoL/L). The dog was hypertensive (SBP 185 mmHg). Amlodipine (Amlodipine; Actavis) and fish oil (Megaderm; Virbac) were added. The dog was treated with a vinblastine/prednisolone protocol, benazepril, amlodipine and

fish oil. He was euthanised 10 months after starting masitinib, due to MCT progression. The dog remained proteinuric (UP:C 0.92–3.31) throughout but albumin normalised, and creatinine remained normal.

Dog-8 had no change in albumin or creatinine when proteinuria was detected at T1 (UP:C 1.27) (albumin T0: 32 g/L; T1: 34 g/L and creatinine T0: 65 µmoL/L; T1: 67 µmoL/L). Masitinib was continued and UP:C was subsequently normal on 2 further occasions (day 26 and 45). Masitinib was discontinued after 47 days due to disease progression and she was euthanised for this reason 29 days later.

Dog-21 developed proteinuria from T1 to T3 (UP:C T0: 0.20; T1: 0.54, T2: 1.06, T3: 0.55) yet serum creatinine and albumin remained stable (creatinine T0: 125 µmoL/L; T1: 110 µmoL/L; T2: 117 µmoL/L; T3: 102 µmoL/L and albumin T0: 34 g/L; T1: 30 g/L; T2: 30 g/L; T3: 28 g/L)). Proteinuria resolved after T3 and never recurred despite the masitinib dose remaining unchanged. A herbal skin supplement (Pomi-T; Herbal medicine) was started at T3. Masitinib was discontinued on day 1028. The dog was euthanised 7 days later due to cardiac disease.

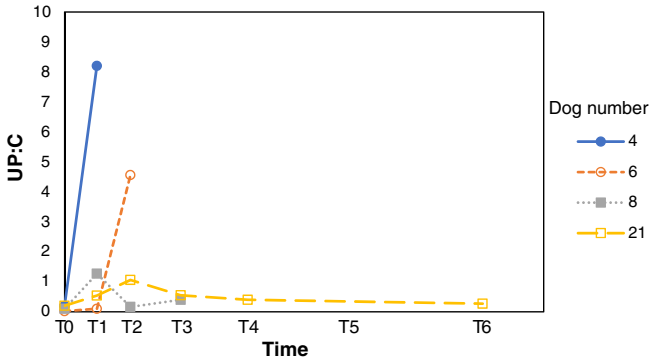

FIG 1. Urine protein to creatinine ratio (UP:C) over time for dogs without proteinuria at baseline that became proteinuric. T0 to T6 – timepoints

Dogs with pre-treatment proteinuria (dogs 23–28)

Two dogs had EL and four had MCT. All dogs with MCT were receiving a histamine-1-receptor antagonist, two a histamine-2-receptor antagonist and one a proton pump inhibitor. Two dogs had comorbidities (dog-23 and dog-26; Table 4) and dog-28 was receiving S-adenosyl methionine (Samylin; VetPlus).

The median pre-treatment UP:C was 1.68 (range: 0.66–4.4). Proteinuria was classified as pathological renal in five dogs (3 were receiving prednisolone). In the sixth (dog-24), proteinuria was classified as pathological renal or post-renal. Creatinine was within the reference interval in all dogs. Three dogs had low albumin (23 g/L, 27 g/L, 28 g/L) (RR: 29 and 36).

Effect of masitinib treatment

Median masitinib starting dose was 11.67 mg/kg (Table 2). Masitinib treatment was discontinued due to lack of efficacy in three dogs, and three were euthanased on treatment, two for disease progression and one for an unknown reason. Gross disease was present in all dogs at all timepoints.

Median UP:Cs were 0.56, 0.56 and 0.29 at T1, T2 and T3, respectively (Fig. 2). Proteinuria was not categorised as pre-renal, physiological renal or post-renal, nor was creatinine increased above the RR in any dog at any timepoint after baseline.

In three dogs (dog-23, dog-24, dog-25), the UP:C magnitude declined at each visit despite starting treatment with masitinib (Table 5, Fig. 2). None of these dogs had reductions in serum albumin during treatment (albumin T0: 23 g/L, 30 g/L and 28 g/L and minimum values: 22 g/L, 29 g/L, 28 g/L, respectively).

The three remaining dogs (dog-26, dog-27, dog-28) had UP:C fluctuations over time but none had increases of over 50%. In two dogs (dog-27 and dog-28), UP:C increases occurred when prednisolone doses were increased. Of these three dogs, one had a 7-day masitinib drug holiday followed by an 11.3% dose reduction (due to neutropenia) and one (dog 28) had a 60% dose reduction (due to hyporexia and diarrhoea). In these three dogs,

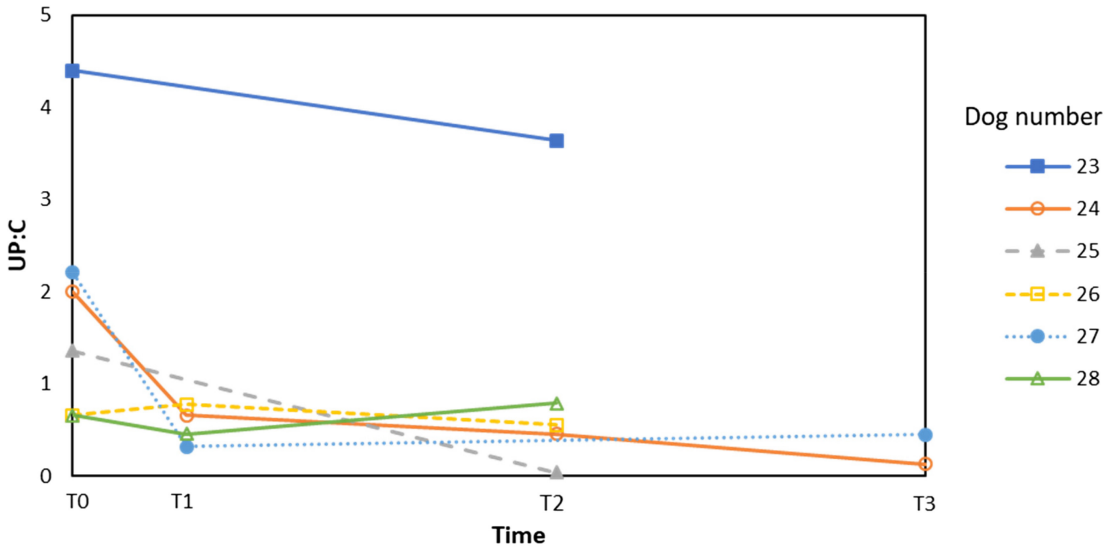

FIG 2. Urine protein to creatinine ratio (UP:C) over time for dogs with proteinuria at baseline. T0 to T3 – timepoints

Table 5. Presence or absence of proteinuria at each timepoint and outcome in dogs with proteinuria at baseline

Dog Number	T0 (baseline)	T1 (days 7 to 20)	T2 (days 21 to 34)	T3 (days 35 to 62)	T4 (days 63 to 90)	T5 (days 91 to 146)	T6 (days 147 to 202)
23	**P**	Missing	**P**	Off masitinib	Off masitinib	Off masitinib	Off masitinib
24	**P**	**P**	NP	NP	Off masitinib	Off masitinib	Off masitinib
25	**P**	Missing	NP	Dead	Dead	Dead	Dead
26	**P**	**P**	NP	Off masitinib	Off masitinib	Dead	Dead
27	**P**	NP	Missing	NP	Dead	Dead	Dead
28	**P**	NP	**P**	Missing	Dead	Dead	Dead

NP Non-proteinuric (UP:C < 0.5), P Proteinuric (UP:C ≥ 0.5)
T0 to T6 – timepoints

albumin declined slightly over time (albumin T0: 30 g/L, 27 g/L and 29 g/L and minimum values 27 g/L, 24 g/L, 28 g/L).

DISCUSSION

Four (18.2%) out of 22 dogs that were non-proteinuric pre-treatment developed proteinuria within 202 days of masitinib commencement. This is similar to the proteinuria incidence of 16 and 23% previously described (Cadot *et al.* 2011, Smrkovski *et al.* 2015). All four of these dogs had developed proteinuria by day 31 (T2).

Little is known about which dogs are predisposed to development of proteinuria with masitinib treatment. Hahn *et al.* (2008) suggested that pre-treatment azotaemia increased the likelihood of masitinib-associated renal disorders including proteinuria; however, none of the dogs in this study had pre-treatment azotaemia. Few dogs developed proteinuria, preventing us drawing any conclusions about other predisposing factors, however pre-treatment urine specific gravity values were lower in those that developed proteinuria (Table 2). This warrants further investigation.

There is limited information about when proteinuria develops in masitinib treated dogs. Cadot *et al.* (2011) suggested that in 202 dogs treated with masitinib, proteinuria tended to develop within 3 months of drug commencement, although no further details were given. Smrkovski *et al.* (2015) reported that all adverse events (including proteinuria) occurred within 6 months despite a median treatment time of 300 days. Proteinuria was not identified for the first time in any dogs in this study after 31 days, despite being treated and monitored for a median of 87 days. Although in two reports of dogs developing severe protein losing nephropathies (PLN) on masitinib therapy, proteinuria was not noted until 5 and 11 weeks following treatment starting, the first only had one urine dipstick test (showing trace protein) performed after 2 weeks and the latter had no earlier urinalysis results reported on treatment (Sum *et al.* 2010; Devine & Polzin 2016). Proteinuria may have been detected earlier in both cases had more frequent testing been performed. Our results suggest proteinuria is more likely to develop within the first month of treatment, emphasising the importance of urine protein monitoring during this period.

Although proteinuria was detected within 16 days of masitinib commencement in 3 dogs, the fourth (dog-6) was non-proteinuric after 2 weeks but developed marked proteinuria and hypoalbuminaemia 2 weeks later. Similarly, in two reports of dogs developing PLN, early urine tests did not identify proteinuria, but severe proteinuria was identified 2 to 3 weeks later (Sum *et al.* 2010, Brown *et al.* 2013). This demonstrates that a normal UP:C early in masitinib treatment does not preclude development of severe proteinuria within the first month.

Proteinuria can be marked, with UP:C > 5 and concurrent severe hypoalbuminaemia (<12 g/L) reported in two dogs (Sum *et al.* 2010, Brown *et al.* 2013). Two dogs developed anuric acute renal failure (ARF) following development of proteinuria on treatment and were subsequently euthanased (Brown *et al.* 2013, Smrkovski *et al.* 2015). Studies that have monitored urinalysis and reported adverse effects in groups of mastinib-treated dogs reported limited information on proteinuria magnitudes and patient outcomes. In one study, proteinuria was categorised as severe (VCOG-CTCAE grade III) in 36.4% of 33 dogs that developed proteinuria (Cadot *et al.* 2011). It is unclear how many dogs developed hypoalbuminaemia or died as a result. Smrkovski *et al.* (2015) reported that two dogs (33.3%) developed VCOG-CTCAE grade I, 3 (50%) VCOG-CTCAE grade II and 1 VCOG-CTCAE grade III proteinuria. In our study, the highest UP:C identified on treatment was 8.2; however, the UP:C continued to increase and hypoalbuminaemia developed shortly after treatment discontinuation. Of the other three dogs that developed proteinuria, UP:C exceeded 2.0 in only one and none developed hypoalbuminaemia. Overall, none of the dogs developed ascites or oedema, azotaemia or were euthanased because of proteinuria; however, UP:Cs were closely monitored, and treatment stopped when UP:C > 2.0. Although proteinuria can be severe and cause fatal complications, this appears to be relatively rare, particularly with frequent monitoring and prompt action should it occur.

When proteinuria is detected in a dog receiving masitinib, decisions must be made as to what action is taken. The datasheet recommends monthly urine dipstick and albumin monitoring, and further testing (including UP:C, creatinine and albumin) if urine protein exceeds 30 mg/dl (AB Science 2009). Temporary treatment discontinuation is advised if UP:C > 2.0 (AB Science 2009). In another reported protocol treatment was discontinued if VCOG-CTAE grade III proteinuria (UPC > 1.0 for ≥14 days) was identified (Hahn *et al.* (2008); Smrkovski *et al.* (2015)). Although temporary or permanent masitinib discontinuation is safest to reduce the risk of development of severe PLN, this must be considered against the reason for drug administration in dogs with potentially life-limiting neoplastic disease.

Various post-renal causes have been reported to increase UP:C to greater than 1.0 and greater than 2.0 (Bagley *et al*. 1991, Vaden *et al*. 2004, Jillings *et al*. 2019). We attempted to retrospectively identify the origin of proteinuria. In two dogs, we could not exclude a post-renal origin of proteinuria. Despite ongoing masitinib treatment in dog-8, UP:C normalised (1.27 to 0.16), possibly due to resolution of post-renal proteinuria. In dog-4, UP:C reduced (8.2 to 2.5) but remained greater than 0.5 one week later despite masitinib discontinuation. Following identification of proteinuria less than 2.0 in dogs receiving masitinib, investigations (including collection of a cystocentesis sample [to confirm persistence and exclude reproductive disease], urine sediment examination and urine culture [to exclude post-renal proteinuria] and haematology and biochemistry profiles [to exclude pre-renal proteinuria]) should be performed to try and exclude non-renal or physiological causes, unrelated to masitinib administration. If these are identified, institution of appropriate treatment may avoid the need for masitinib therapy interruption.

If renal proteinuria is still suspected, a decision must be made whether to discontinue therapy. Patient outcomes when masitinib has been continued despite the development of proteinuria have been variable. In the report by Brown *et al*. (2013), masitinib was continued despite the detection of proteinuria (UP:C 0.6) and 2 weeks later severe proteinuria (UP:C 20.8) and concurrent severe hypoalbuminaemia occurred. Treatment was then discontinued but anuric ARF developed and the dog euthanased within 3 days. However, in the study by Smrkovski *et al*. (2015) in which monthly urine monitoring was performed, five dogs receiving masitinib developed low magnitude proteinuria (0.5–1.0) or UPC greater than 1.0 for less than 14 days. In those cases, treatment was continued but neither grade III proteinuria nor serious complications occurred. Similarly, in this study, both dogs which developed UP:Cs between 1.0 and 2.0 did not develop significant worsening of UP:C, hypoalbuminaemia or azotaemia despite continued masitinib treatment. In both, UP:C was normal 13 and 75 days later and remained so throughout treatment (a further 20 and 939 days). This suggests some dogs developing UP:C 0.5–2.0 progress to severe disease and others do not. As we cannot predict which cases will progress and because proteinuria can progress rapidly, if treatment is continued despite low magnitude proteinuria development, repeat monitoring should be performed within 1 week enabling rapid treatment discontinuation if UP:C exceeds 2.0.

Despite masitinib discontinuation, proteinuria can continue to progress rapidly, hypoalbuminaemia and azotaemia may develop or worsen. In one of two dogs (dog-6) with UP:C > 2.0, UP:C worsened markedly (4.56 to 15) and serum albumin fell dramatically (21 to 13 g/L) within 4 days following treatment discontinuation before improving 4 days later. Similarly, in the reports by Sum *et al*. (2010) and Devine & Polzin (2016) in which masitinib was discontinued within 2 days of proteinuria or hypoalbuminaemia documentation, initial worsening of hypoalbuminaemia and/or azotaemia or UP:C occurred prior to improvement. In both cases, complete recovery (except an ongoing UP:C elevation in 1 dog) occurred without long-term ill effects (Sum *et al*. 2010, Devine & Polzin 2016). Not all dogs recover after treatment discontinuation (Smrkovski *et al*. 2015). Recovery can take 1 month and can have negative effects on quality of life and be expensive (Devine & Polzin 2016). Earlier detection and treatment discontinuation appear to lead to quicker recovery, with patients with asymptomatic proteinuria recovering faster than those with clinical signs or sequelae (Sum *et al*. 2010, Devine & Polzin 2016). This supports frequent, possibly weekly, monitoring of UP:C for at least the first month of treatment to allow early discontinuation and make a rapid recovery more likely.

According to the masitinib datasheet, its use is contraindicated in dogs with a pre-treatment UP:C > 2.0 (AB Science 2009). The large studies reporting masitinib treatment do not report pre-treatment UP:C, nor do they specify criteria for exclusion based on pre-treatment UP:C (Hahn *et al*. 2008, Cadot *et al*. 2011, Smrkovski *et al*. 2015, Grant *et al*. 2016). The effect of masitinib administration to dogs with pre-existing proteinuria has not been reported.

Fifteen percent of dogs presented to an oncology service had a UP:C greater than 0.5, and prednisolone, which is commonly administered to patients with neoplasia, can cause proteinuria (Waters *et al*. 1997, Prudic *et al*. 2018). Some dogs in which masitinib treatment may be considered will therefore have pre-treatment proteinuria. We report the renal effects of masitinib therapy in six dogs with pre-treatment proteinuria (median UP:C 1.68). Two dogs had a pre-treatment UP:C > 2.0. Overall, 21% had pre-treatment proteinuria which is similar to the previously reported incidence (Prudic *et al*. (2018)). In five of six dogs pathological renal proteinuria was considered the likely cause. The median time between diagnosis and treatment commencement of 106.5 days and that five of six dogs had had prior treatment suggests that masitinib was selected when other treatment modalities had been exhausted. Masitinib therapy was initiated within the recommended dose range in five of six patients.

In three dogs (including the dog with possible post-renal proteinuria), proteinuria decreased at each subsequent measurement despite masitinib treatment. In the other three, UP:C increases were only present at one timepoint and were all <50%. In two of three dogs with UP:C increases, prednisolone was added, or the dose increased and the masitinib dose reduced at the previous visit suggesting masitinib treatment was not the likely cause. No dogs had masitinib stopped due to proteinuria, nor did any develop hypoalbuminaemia or azotaemia. Although, treatment duration was short (median 32 days), proteinuria developed within 1 month in the non-proteinuric dogs so severe worsening of proteinuria might also be expected in proteinuric patients within this timeframe. This suggests masitinib treatment in dogs with pre-existing proteinuria might be safe, even if a renal origin cannot be excluded. The number of dogs described is small, most dogs had pre-treatment UP:C less than 2.0 and the origin of proteinuria was unknown, but further investigation of masitinib treatment in a larger group of dogs with pre-treatment proteinuria, with longer treatment duration and follow-up, is warranted to enable the safety of its use in these patients to be further evaluated.

This study has certain limitations, including small case numbers, variety of tumours, variable masitinib doses (including

significant dose reductions in some patients due to other toxicities) and concurrent drugs administered. In addition, its retrospective nature resulted in missing data points, no control group, unknown locations of urine sample collection and unconfirmed origins of proteinuria (due to infrequent urine cultures and absence of renal biopsies). More rigorous exclusion of post-renal causes would have been desirable. Routine SBP monitoring was not performed so no comment can be made on the role of hypertension in proteinuria development.

A larger, prospective study of dogs receiving masitinib treatment, including a control population, and in which patients are followed up for longer is now warranted. Patients developing proteinuria should be rigorously investigated to exclude non-renal causes. This should allow the timing of and reasons for development of proteinuria to be confirmed and allow recommendations on the monitoring of proteinuria in these patients to be optimised.

The use of masitinib in dogs in this study was off-licence as C-KIT MCT expression was not determined and many dogs were treated for other tumours.

In this study, the reported incidence of proteinuria with masitinib treatment was similar to that previously reported. Proteinuria only developed within the first month of treatment. Severe proteinuria (UPC > 2.0) was identified in two of four dogs that developed proteinuria. Despite rapid treatment discontinuation in both, proteinuria continued to worsen in one dog prior to UP:C improvement and resolution of sequelae. Weekly UP:C monitoring for the first month of treatment is recommended to facilitate rapid treatment discontinuation when UP:C exceeds 2.0. Development of mild proteinuria (UP:C < 2.0) may not require treatment discontinuation but investigation of its origin and repeat testing within 1 week is recommended.

An additional finding was that significant worsening of proteinuria and development of sequelae did not occur in any of the six dogs with pre-treatment proteinuria; however, further investigation treating these patients is now needed.

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Conflict of interest

No conflicts of interest have been declared.

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