

Metronomic administration of chlorambucil for treatment of dogs with urinary bladder transitional cell carcinoma

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Objective—To determine the antitumor effects and toxicoses of metronomic oral administration of a low dose of chlorambucil in dogs with transitional cell carcinoma (TCC).

Design—Prospective clinical trial.

Animals—31 client-owned dogs with TCC for which prior treatments had failed or owners had declined other treatments.

Procedures—Chlorambucil (4 mg/m², PO, q 24 h) was administered to dogs. Before and at scheduled times during treatment, evaluations of dogs included physical examination, CBC, serum biochemical analyses, urinalysis, thoracic and abdominal imaging including cystosonography for measurement of TCCs, and grading of toxicoses.

Results—29 of 31 dogs had failed prior TCC treatment. Of the 30 dogs with available data, 1 (3%) had partial remission ($\geq 50\%$ reduction in tumor volume), 20 (67%) had stable disease ($< 50\%$ change in tumor volume), and 9 (30%) had progressive disease ($\geq 50\%$ increase in tumor volume or development of additional tumors); 1 dog was lost to follow-up. The median progression-free interval (time from the start of chlorambucil treatment to the day progressive disease was detected) for the dogs was 119 days (range, 7 to 728 days). The median survival time of dogs from the time of the start of chlorambucil treatment was 221 days (range, 7 to 747 days). Few toxicoses were detected; chlorambucil administration was discontinued because of toxicoses in only 1 dog.

Conclusions and Clinical Relevance—Metronomic administration of chlorambucil was well tolerated, and 70% of dogs had partial remission or stable disease. Metronomic administration of chlorambucil may be a treatment option for dogs with TCC. (*J Am Vet Med Assoc* 2013;242:1534–1538)

Transitional cell carcinoma is the most common cancer of the urinary tract in dogs.¹ Treatment typically includes administration of piroxicam alone (a nonspecific COX inhibitor), mitoxantrone and piroxicam, or vinblastine^{1–3}; other treatments for TCC in dogs include carboplatin and piroxicam,⁴ intravesical chemotherapy with mitomycin C,⁵ or cisplatin and piroxicam.^{1,6} Although these treatments can induce remission of TCC and prolong a good quality of life for dogs, TCCs typically become resistant to the drugs over time, and the dogs subsequently die from the cancer.

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ABBREVIATIONS

CI	Confidence interval
COX	Cyclooxygenase
HR	Hazard ratio
MST	Median survival time
PFI	Progression-free interval
TCC	Transitional cell carcinoma

Other treatments are being investigated to improve outcomes and prolong a good quality of life for animals with tumors. One of these treatments is metronomic chemotherapy, during which low doses of chemotherapeutic drugs are repetitively administered to patients to prevent or delay progression of cancer.^{7–10} Metronomic chemotherapy is well tolerated by rodents and humans and is effective for slowing growth of tumors, including tumors for which other treatments have been unsuccessful.^{8,9} Although remission is not typically expected to develop with metronomic chemotherapy, such treatment may help control cancer in animals for a long period of time (ie, cancer is treated as a chronic disease that is managed, rather than cured). Although metronomic chemotherapy has not been evaluated for the treatment of TCC in dogs, to the authors' knowledge, it has been used for treatment of other tumors in dogs.^{10–13} Metronomic chemotherapy may have effects

via multiple mechanisms, including inhibition of the formation of new blood vessels in tumors and modulation of immune system function.^{7,14–17}

Chlorambucil is an orally administered alkylating drug.¹⁸ This drug was selected for evaluation as a metronomic chemotherapeutic agent for the treatment of dogs with TCC in the study reported here because alkylating agents are effective treatments for experimentally induced tumors in rodents when administered in metronomic schedules,^{7,8} chlorambucil is available in an oral formulation,¹⁹ chlorambucil is well tolerated by dogs (even at high doses),^{19–21} and metronomic administration of chlorambucil has been successful for treatment of dogs with other types of cancer.¹³ The hypothesis was that metronomic administration of chlorambucil would delay the progression of TCC and be well tolerated by dogs.

Materials and Methods

Animals—Thirty-one client-owned dogs with TCC treated at the Purdue University Veterinary Teaching Hospital were enrolled in the study. The sample size (≥ 30 dogs) was determined on the basis of the number of dogs needed to estimate tumor response to treatment.²² Dogs had naturally occurring, histopathologically confirmed, measurable (ie, measurable TCC lesions in the bladder) urinary bladder TCC. Only dogs for which prior treatments for TCC failed to control the cancer and those for which owners had declined other treatments were included in the study. Prior treatments were determined to have failed on the basis of a $\geq 50\%$ increase in tumor volume or the development of additional tumors during treatment. Dogs that had previously received COX inhibitors were allowed to continue to receive those drugs for treatment of pain during this study only if TCC had been progressing despite COX inhibitor treatment. Written informed consent was obtained from owners for inclusion of dogs in the study. The study was approved by the Purdue Animal Care and Use Committee.

Clinical trial—Dogs were enrolled in a prospective clinical trial during which TCC was treated via metronomic administration of chlorambucil. The primary endpoints evaluated were tumor response (ie, remission, stable disease, or progressive disease) and PFI.² Remission of tumors or a lack of detectable tumor growth for ≥ 8 weeks was considered a positive response to treatment, because cancer progression is typically detected within 4 to 8 weeks after initiation of unsuccessful treatments for dogs with TCC,^{1,23} and dogs that undergo surgical debulking of tumors alone typically have a doubling of TCC volume within 4 to 8 weeks.¹

Dogs received chlorambucil^a (approx 4 mg/m², PO, q 24 h). That chlorambucil dose was selected because it is less than the dose commonly administered to dogs (8 mg/m²) and is likely to be less than the maximum tolerated dose of the drug for such animals,²⁴ and results in serum concentrations that are not directly cytotoxic to TCC cells in vitro (unpublished data). In addition, that dose is well tolerated and has antitumor activity in dogs.¹³ For dogs that weighed ≤ 8.0 kg (17.6 lb), chlorambucil was compounded by commercial compounding pharmacies to allow admin-

istration of a dose of 4 mg/m². For dogs that weighed > 8.0 kg, the chlorambucil dose was rounded to the nearest dose that could be administered with whole 2-mg tablets; dogs that weighed 8.1 to 20.0 kg (17.8 to 44.0 lb) received 2 mg of chlorambucil, dogs that weighed 20.1 to 40.0 kg (44.2 to 88 lb) received 4 mg of chlorambucil, and dogs that weighed 40.1 to 60.0 kg (88.2 to 132 lb) received 6 mg of chlorambucil every 24 hours. Toxicoses were graded with the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events.²⁵

Dogs were evaluated prior to and at 4- to 6-week intervals during chlorambucil treatment. Evaluations for each dog included performance of a physical examination (including digital rectal examination), CBC, serum biochemical analyses, urinalysis, thoracic radiography, abdominal radiography, abdominal ultrasonography, and cystosonography. Cystosonography was performed via a method similar to that described in another report²⁶ with 2 modifications: the amount of saline (0.9% NaCl) solution infused in the bladder was approximately 4 to 6 mL/kg (depending on bladder distensibility), and female dogs with extensive urethral TCC were not catheterized because of the risk of urethral perforation (such dogs were confined for a short period to allow the bladder to fill with urine). Bladder distention was evaluated via cystosonography so that the amount of urinary bladder distention for each visit was similar. Cystosonography was performed for all dogs at all evaluation times by 1 investigator (DWK). Sizes of metastases in dogs detected via radiography or ultrasonography were recorded for each evaluation time. The TCC stages were determined with the World Health Organization tumor-node-metastasis (ie, TNM) classification method.²⁷

Classification of tumor response to treatment—Tumor responses to treatment were classified as complete remission, partial remission, stable disease, or progressive disease. Complete remission was defined as complete resolution of all tumor masses in a dog. Partial remission was defined as a $\geq 50\%$ reduction in tumor volume with no additional tumors detected. Stable disease was defined as a $< 50\%$ change in tumor volume with no additional tumors detected. Progressive disease was defined as a $\geq 50\%$ increase in tumor volume or the development of additional tumors. Dogs with stable disease or remission continued to receive treatments. Dogs with progressive disease were eligible to receive treatments other than chlorambucil off study.

The PFI was defined as the time from the first day of treatment with chlorambucil to the day a dog was determined to have progressive disease. Survival time was defined as the time from the first day of treatment with chlorambucil to the day a dog died.

Statistical analysis—Median PFIs and survival times were calculated with Kaplan-Meier curves. Stratified Cox proportional-hazard analysis with forward stepwise regression was used for analysis of data for the variables age, at-risk breeds for TCC (Scottish Terrier, West Highland White Terrier, Shetland Sheepdog, Wire Fox Terrier, and Beagle), sex, adverse effects of chlorambucil treatment, prior treatments (including surgery, COX inhibitors, or chemotherapy), type of

COX inhibitor administered, TNM stage, urethral involvement, doxycycline treatment (for treatment of *Mycoplasma* spp infection of urinary tracts), and time of detection of another tumor type relative to PFI and survival time. Data for 2 dogs that were alive at the time of the last follow-up evaluation were included in the analysis as censored data. The stratified Cox proportional-hazard analysis model assumptions were evaluated for goodness of fit; the model assumptions were not violated. Values of $P < 0.05$ were considered significant. Statistical analysis was performed with commercially available software.^b

Results

Thirty-one dogs were enrolled in the study between January 2007 and April 2010. Twenty-one spayed female, 1 sexually intact female, and 9 neutered male dogs were included in the study. Seven Scottish Terriers, 7 mixed-breed dogs, 5 Shetland Sheepdogs, 4 Beagles, and 1 each of West Highland White Terrier, Wire Fox Terrier, Golden Retriever, Bernese Mountain Dog, Shih Tzu, Labrador Retriever, Miniature Pinscher, and a Treeing Walker Coonhound were included in the study. The median weight of the dogs was 11.7 kg (25.7 lb; range, 4.5 to 33.1 kg [9.9 to 72.8 lb]). The median age of the dogs at the time of enrollment in the study was 11 years (range, 5 to 14 years). Tumor stages were determined to be $T_2N_0M_0$ for 19 dogs, $T_2N_1M_0$ for 1 dog, $T_2N_0M_1$ for 6 dogs, $T_2N_1M_1$ for 2 dogs, $T_3N_0M_0$ for 2 dogs, and $T_3N_0M_1$ for 1 dog. Ten dogs had metastases, and 12 dogs had urethral TCC involvement.

Twenty-nine dogs had received prior treatments for TCC including piroxicam ($n = 13$ dogs), deracoxib (10), firocoxib (10), mitoxantrone (7), surgery (5), intravesical chemotherapy with mitomycin C (5), cyclophosphamide (4), carboplatin (3), 5-azacitidine (3), vinblastine (3), doxorubicin (2), and cisplatin (1) before enrollment in the study. Of the 28 dogs that had failed treatment with a COX inhibitor prior to enrollment, 25 continued to receive that drug during the study to reduce signs of pain attributable to TCC or comorbid conditions. Twelve dogs received other treatments (vincristine, vinblastine, deracoxib, firocoxib, mitoxantrone, or carboplatin) after metronomic administration of chlorambucil had failed.

Of the 30 dogs included in the study for which data were available, 1 (3%) had partial remission, 20 (67%) had stable disease, and 9 (30%) had progressive disease; 1 dog was lost to follow-up. Median PFI of the dogs was 119 days (range, 7 to 728 days; Figure 1). The MST of the dogs was 221 days (range, 7 to 747 days; Figure 2).

The PFI was negatively affected by the TNM stage of the TCC. Dogs with metastasis were 3.71 times as likely to have tumor progression as those without metastasis (HR, 3.71; 95% CI, 1.02 to 13.55; $P = 0.046$). Survival time was negatively affected by the presence of distant metastases of TCCs (HR, 10.90; 95% CI, 2.73 to 43.50; $P = 0.001$), prior treatment with a COX inhibitor (HR, 232.54; 95% CI, 14.55 to 3,717.50; $P < 0.001$), prior treatment with firocoxib (HR, 39.80; 95% CI, 5.45 to 290.53; $P < 0.001$), prior treatment with

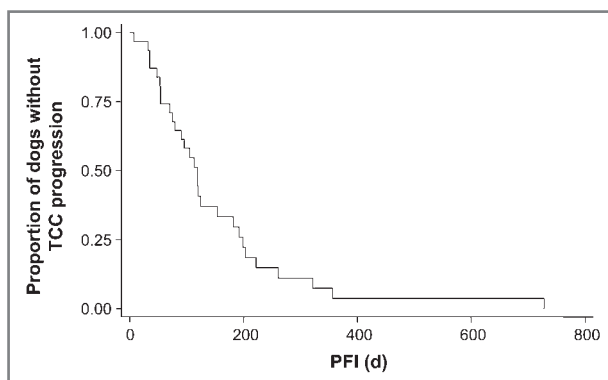


Figure 1—Kaplan-Meier curve of PFI for 31 dogs with TCC that were treated via metronomic administration of chlorambucil.

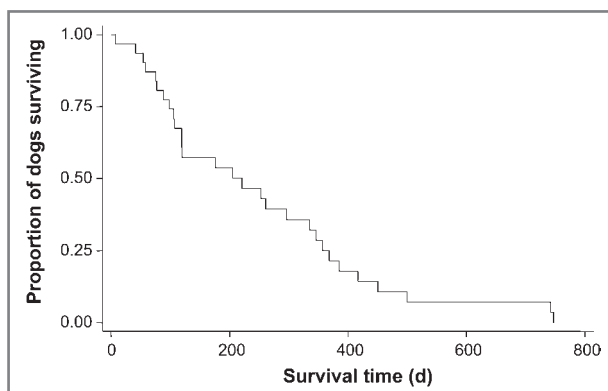


Figure 2—Kaplan-Meier curve of survival time for the dogs in Figure 1.

piroxicam (HR, 7.94; 95% CI, 1.81 to 34.68; $P = 0.006$), at-risk breed status (HR, 6.10; 95% CI, 1.59 to 23.34; $P = 0.008$), and urethral TCC involvement (HR, 3.19; 95% CI, 1.03 to 9.89; $P = 0.045$). Survival time was positively affected by detection of another tumor type (HR, 0.05; 95% CI, 0.01 to 0.37; $P = 0.003$) and prior treatment with doxycycline (HR, 0.25; 95% CI, 0.07 to 0.89; $P = 0.032$).

Few toxicoses attributable to chlorambucil treatment were detected in dogs. Four dogs had gastrointestinal tract adverse effects (2 dogs had diarrhea and 2 had anorexia [Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events grade 1]). Two dogs had lethargy (grade 1). One dog had anemia (grade 2), neutropenia (grade 2), and thrombocytopenia (grade 3) after 20 months of chlorambucil treatment; these problems resolved and the dog lived for several months after discontinuation of chlorambucil administration.

Discussion

The most important finding of the present study was that most (21/30 [70%]) dogs that underwent metronomic administration of chlorambucil and for which data were available had stable disease or partial remission of TCC, even though 29 of 31 (94%) dogs included in the study had received prior treatments that had become ineffective. The percentage of dogs with partial remission or stable disease after metronomic chemotherapy with chlo-

rambucil in the present study was similar to that for dogs with TCC in other studies that were treated with piroxicam alone (71%),¹ mitoxantrone and piroxicam (81%),³ or carboplatin and piroxicam (83%)⁴; most dogs in those other studies had not received prior treatments. The finding of the present study that the MST for dogs that had previously failed other treatments was > 7 months after the start of the study suggested metronomic chemotherapy with chlorambucil was beneficial for treatment of dogs with TCC.

The inclusion of an untreated control group of dogs would have been helpful for interpretation of PFI and survival time results of the present study. Untreated dogs were not included because we considered withholding of treatments from dogs to be unacceptable. Similarly, dogs that underwent standard chemotherapeutic drug protocols were not included in this study because the goal of the study was not to compare results for metronomic chlorambucil chemotherapy with those for other chemotherapeutic protocols; the goal of the study was to determine whether metronomic chemotherapy with chlorambucil was beneficial for treatment of dogs that had failed prior treatments for TCC and those for which owners had declined treatment with other chemotherapeutic drugs. The PFI and survival time results for dogs in the present study were comparable to results for dogs with TCC that received other orally administered treatments in 2 other studies.^{28,29} In one of those other studies,²⁸ 34 dogs received piroxicam alone for treatment of TCC; the median PFI for those dogs was 135 days, and the MST was 181 days. Of the 34 dogs in that other study, 28 had not received prior treatments, and 29 did not receive other drugs after cancer progression was detected with piroxicam treatment. In another study,²⁹ 26 dogs with TCC received deracoxib alone; the median PFI for those dogs was 133 days, and the MST was 323 days. The median PFI (119 days) and survival time (221 days) for dogs that received chlorambucil in the present study were similar to results of those other studies.^{28,29} In addition, the MST of 55 dogs that underwent debulking of TCCs alone in another study (conducted before medical treatment protocols for TCC were determined) was 109 days.²³ Although the PFI was not determined for all dogs in that study, progression of cancer was detected in many of the dogs within 4 to 8 weeks after surgery. In addition, detection of control of cancer for 3 of 10 dogs with TCC metastases that were receiving chlorambucil was encouraging because the presence of metastases is a negative prognostic factor in dogs treated for TCC.²³ One of the dogs in the present study that had metastases had a PFI of 199 days and a survival time of 450 days. That dog continued to receive chlorambucil until it died, and no other chemotherapeutics were administered.

Another important finding of the present study was that metronomic administration of chlorambucil was well tolerated by dogs. Only 7 of the 30 (23%) dogs had toxicoses, and those toxic effects were typically mild. Only 1 dog had a grade 3 toxicosis, and that was not detected until 20 months after chlorambucil treatment was started.

Chlorambucil has several advantages for metronomic administration including a small tablet size, rapid absorption, and few reported adverse effects.^{18,20,21} Another

alkylating agent, cyclophosphamide, has been used in metronomic administration protocols for dogs, but that drug can cause hemorrhagic cystitis.^{11,12} Differentiation of clinical signs caused by TCC and those caused by cystitis in dogs can be difficult. Therefore, chlorambucil may be a better choice than cyclophosphamide for metronomic chemotherapy in dogs with TCC.

In order to determine the antitumor effects and toxicoses of metronomic administration of chlorambucil for dogs, all dogs included in this study had measurable TCCs, and none of them received other chemotherapeutic drugs, surgery, or radiation treatments concurrently. Dogs that had TCC progression during prior COX inhibitor drug treatment were allowed to continue to receive COX inhibitors if necessary to relieve signs of pain attributable to TCC or concurrent arthritis. Cyclooxygenase inhibitor drugs are effective for treatment of mild to moderate signs of pain caused by cancer, arthritis, and other associated conditions in animals.³⁰ Cyclooxygenase inhibitors can enhance the activity of other chemotherapeutic drugs.²³ In the present study, administration of COX inhibitor drugs was not started at the same time as the initiation of chlorambucil treatment, and administration of COX inhibitors was only permitted for dogs in which TCC had progressed despite such treatment. Further studies would be needed to determine whether initiation of COX inhibitor treatment concurrent with initiation of chlorambucil administration would enhance the antitumor effects of metronomic chemotherapy.

Several factors were associated with PFIs and survival times of dogs undergoing metronomic chemotherapy with chlorambucil in the present study. Survival time was significantly longer for dogs that developed an additional type of cancer than it was for dogs with TCC as the sole type of cancer; dogs with additional types of cancer were likely dogs that survived long enough to develop a second tumor type. A second tumor type would not be expected to confer a survival advantage for dogs. Dogs that received doxycycline as a treatment for *Mycoplasma* infection of the urinary tract had a longer survival time than dogs that did not receive that treatment in the present study. Doxycycline can have antiangiogenic effects.³¹ Seven dogs in the present study received doxycycline, and that drug was not administered to any of the dogs for longer than 1 month. It was unknown whether the effects of doxycycline on survival time of dogs in this study were attributable to antiangiogenic effects or other causes.

Variables that were negatively associated with survival time of dogs in the present study included detection of metastases at distant sites, prior administration of COX inhibitor drugs, prior administration of piroxicam, prior administration of firocoxib, urethral TCC involvement, and an at-risk breed status. Results of another study¹ indicate that metastasis and urethral TCC involvement is associated with a shorter survival time versus dogs without such findings. Only 3 dogs in the present study had not previously received a COX inhibitor drug; therefore, it was difficult to determine conclusions regarding the effects of such treatments on survival time. Of the 28 dogs that had received a COX inhibitor drug for treatment of TCC prior to the start

of the study, all but 5 had received piroxicam or firocoxib. Therefore, as for the results regarding treatment with any type of COX inhibitor drug, the negative effects of firocoxib and piroxicam treatment on survival of dogs may have been attributable to the number of dogs receiving each of those types of COX inhibitor drugs. The reason that at-risk breed status was associated with a shorter survival time of dogs versus dogs of other breeds in this study could not be determined. This finding may be attributable to the fact that such dogs had typically received prior treatments. Breed has not previously been associated with the response of dogs with TCC to other drugs.²³

Results of the present study indicated that low-dose metronomic administration of chlorambucil was well tolerated by dogs and such treatment can slow TCC progression and prolong survival for several months after other treatments have failed. Metronomic administration of chlorambucil may be a treatment option for dogs with TCC that had failed other treatments with other chemotherapeutic drugs or for dogs with owners who have declined such treatments. Metronomic administration of chlorambucil may be easier and have a lower cost than other injectable chemotherapeutic drug protocols.

- a. Leukeran, GlaxoSmithKline, Philadelphia, Pa.
- b. STATA software, version 10.2, StataCorp, College Station, Tex.

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