Toxic effects and antitumor response of gemcitabine in combination with piroxicam treatment in dogs with transitional cell carcinoma of the urinary bladder

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Objective—To investigate whether combined treatment with gemcitabine and piroxicam in dogs with transitional cell carcinoma (TCC) of the urinary bladder is tolerated and provides an advantage in terms of survival time over previously reported treatments.

Design—Clinical trial.

Animals—38 dogs with TCC of the urinary bladder.

Procedures—Dogs were treated with gemcitabine (800 mg/m², IV over 30 to 60 minutes, q 7 d) and piroxicam (0.3 mg/kg [0.14 mg/lb], PO, q 24 h). Complete blood cell counts were monitored prior to each gemcitabine treatment. All toxic effects of gemcitabine in dogs were recorded. Primary tumors were ultrasonographically reevaluated after 4 gemcitabine treatments.

Results—Dogs received a median of 8 gemcitabine treatments (range, 1 to 38 treatments/dog). In response to treatment, 10 of 38 (26.3%) dogs had grade 1 gastrointestinal tract signs, 11 (28.9%) had grade 2, and 5 (13.2%) had grade 3. Grade 1 neutropenia developed in 6 (15.8%) dogs and grade 2 and 3 neutropenia in 2 (5.3%) dogs each. Thrombocytopenia was rare. All dogs had improvement of clinical signs of disease. Two dogs had a complete tumor response, 8 had a partial response, 19 had stable disease, and 8 had progressive disease. Median survival time with treatment was 230 days.

Conclusions and Clinical Relevance—Administration of gemcitabine in combination with piroxicam treatment failed to provide a longer overall survival time in dogs with TCC of the urinary bladder, compared with previously reported treatment strategies. However, this combination of chemotherapy did provide a new treatment alternative with fewer adverse effects. (J Am Vet Med Assoc 2011;238:1004–1010)

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determined. In 1 study,13 healthy Beagles received intravesical administration of gemcitabine with no development of adverse effects. However, high doses > 1,000 mg/m² resulted in bone marrow hypoplasia, cystitis, and intestinal necrosis.13 In a subsequent study,14 dogs had greater myelosuppression when gemcitabine was administered as an IV 30-minute infusion instead of an IV bolus.12 However when gemcitabine was given biweekly to dogs with solid malignant tumors or lymphoma at a dose of 300 to 675 mg/m², minimal adverse effects were reported.24 More recently, gemcitabine given to dogs with mammary tumors at the dosage of 800 mg/m² every 7 days was well tolerated.25

On the basis of these considerations, the purpose of the study reported here was to investigate whether combined treatment with gemcitabine and piroxicam in dogs with TCC of the urinary bladder is tolerated and provides an advantage in terms of survival time over previously reported treatment protocols. We prospectively investigated the combination of gemcitabine and piroxicam in dogs with TCC of the urinary bladder to define whether the adopted protocol was tolerated and effective.

Materials and Methods

Patient selection—Dogs with cytologically or histopathologically confirmed TCC of the urinary bladder were eligible for entry into this bi-institutional study. All owners were informed of the investigational nature of the study, and written informed consent was obtained. The study was performed in compliance with institutional guidelines for research on animals. Cytologic samples were obtained by use of ultrasound-guided traumatic catheterization; a diagnosis of TCC was formulated if clusters of epithelial cells that had criteria of malignancy (including anisocytosis, anisokaryosis, pleomorphic nuclei, variably sized nucleoli, and variable nuclear-to-cytoplasmic ratio) were observed.

Additional requirements for inclusion in the study included the following: no prior chemotherapy or radiation therapy within 3 weeks of study entry; adequate bone marrow function, as evidenced by neutrophil counts of ≥ 3,000 cells/µL, platelet counts of ≥ 100,000 platelets/µL, PCV ≥ 30%; presence of measurable disease; and receipt of ≥ 1 dose of gemcitabine in dogs. Patients were removed from the study if they had disease progression, if they had unacceptable adverse effects, or if the owner requested the dog’s withdrawal.

Before entering the study, all dogs underwent complete staging, including CBC with differential, serum biochemical analysis, urinalysis, abdominal ultrasonography, and thoracic radiography, and were staged accordingly to the World Health Organization’s TNM classification.15 Tumor size was defined as T₁ (superficial papillary tumor), T₂ (tumor invading the bladder wall with induration), or T₃ (tumor invading neighboring organs). Lymph node status was defined as N₀ (no evidence of regional lymph node involvement), N₁ (regional lymph nodes involved), or N₂ (regional and juxta-regional lymph nodes involved). Metastasis status was defined as M₁ (no evidence of distant metastasis) or M₂ (distant metastasis detected).

Tumor measurement was performed by means of ultrasonography by the same radiologist. Owners were instructed to offer water ad libitum and not to allow their dogs to urinate for 1 hour before the ultrasonographic examination. To obtain consistent 2-D measurements over multiple examinations, dogs were always positioned in dorsal recumbency, and the bladder was fully distended, as evidenced by a urinary bladder wall of < 2 mm in thickness.34

Treatment—The starting dose of gemcitabine was 800 mg/m² IV, every 7 days. Gemcitabine was reconstituted by adding 5 mL of saline (0.9% NaCl) solution. A concentration of 38 mg/mL was obtained. Reconstituted drug was then added to a 100-mL bag of saline solution and administered over a 30- to 60-minute period via an indwelling catheter placed in a peripheral vein. In addition to gemcitabine, all dogs were concurrently receiving piroxicam (0.3 mg/kg [0.14 mg/lb], PO, q 24 h). Clavulanate-potentiated amoxicillin (25 mg/kg [11.35 mg/lb], PO, q 12 h) was administered for 7 days after each of the first 4 gemcitabine treatments, then only if needed thereafter.

During the study, dogs were evaluated weekly as outpatients for clinical assessment. Any improvement in clinical signs of disease (as reported by the owners) and toxic effects of gemcitabine in combination with piroxicam treatment were recorded. A CBC was performed followed by administration of gemcitabine. Serum biochemical variables were measured every 4 weeks or more frequently if clinically indicated. Toxicity of gemcitabine in dogs was evaluated on the basis of the history obtained from the owner, physical examination findings, and clinicopathologic data and graded according to the Veterinary Cooperative Oncology Group.35 This system was applied retrospectively to categorize documented adverse effects, as the criteria were published after some of the dogs had already been treated. A 25% dose reduction with no treatment delay was performed in dogs with a neutrophil count of < 2,000 cells/µL (reference range, 3,000 to 12,000 cells/µL). When myelosuppression persisted, dogs were retreated 3 to 7 days later and treated with gemcitabine at the time that the CBC was within reference range limits. In the dogs for which treatment was delayed, intervals were increased to 10 to 14 days depending on the severity of myelosuppression.

Response and follow-up assessment—Ultrasonographic examination of the entire abdomen was performed after the fourth administration of gemcitabine and every 3 weeks thereafter to assess both local antitumor response and possible development of regional or distant metastatic disease. Measurements of the primary tumor were compared with that obtained during the previous ultrasonographic examination. Antitumor responses were assessed according to standard criteria. Complete response was defined as the disappearance of all detectable tumors, partial response as a reduction of ≥ 50% in tumor volume, stable disease as ≤ 25% in total tumor volume, and progressive disease as an increase of ≥ 25% in total tumor volume or the appearance of metastasis. All antitumor responses were required to last
by use of a commercially available software program. The frequency of treatment (ie, chemotherapy or surgery) previous to univariate logistic regression analysis. The frequency of development or lack thereof of adverse effects on the bone marrow or gastrointestinal tract was studied with use of the Cox proportional hazard model. Dogs were censored if they were still alive, were lost to follow-up, or died as a result of causes unrelated to TCC. The effect of body weight, considered as a continuous variable, on presence or absence of adverse effects on the bone marrow and gastrointestinal tract. Influences of these factors on survival time were evaluated by use of the Kaplan-Meier product-limit followed by log rank test. Factors that had values of P < 0.15 on univariate analysis were further used to evaluate their independence by use of the Cox proportional hazard model. Dogs were censored if they were still alive, were lost to follow-up, or died as a result of causes unrelated to TCC. The effect of body weight, considered as a continuous variable, on presence or absence of adverse effects on the bone marrow or gastrointestinal tract was studied with use of Kaplan-Meier hazard regression analysis. The frequency of treatment (ie, chemotherapy or surgery) previous to study admission was compared in dogs with progressive disease and those with other responses by use of the Fisher exact test. A value of P < 0.05 was used to indicate significance. Statistical analysis was conducted by use of a commercially available software program.

**Results**

**Patient population**—Thirty-eight dogs with TCC of the urinary bladder were treated. In 30 of the 38 (78.9%) dogs, the diagnosis of TCC was formulated on the basis of cytologic findings, whereas in the remaining 8 (21.1%) dogs, the diagnosis was reached by means of histologic examination.

Median age was 11 years (mean, 10.5 years; range, 5 to 15 years). Twenty of 38 (52.6%) dogs were males (17 neutered and 3 sexually intact), and 18 (47.4%) were spayed females. In addition to the urinary bladder, 5 of 20 males also had prostatic involvement, and of the 18 females, 7 also had urethral involvement. Thirty-seven of 38 (97.4%) dogs had trigonal involvement. There were 12 mixed-breed dogs. Purebred dogs included the following: Shetland Sheepdog (n = 6), Beagle (2), West Highland White Terrier (2), Scottish Terrier (2), Poodle (2), and 1 each of Bull Terrier, Old English Sheepdog, American Eskimo Dog, Australian Terrier, Pug, English Springer Spaniel, American Staffordshire Terrier, Airedale Terrier, Labrador Retriever, Alaskan Malamute, Golden Retriever, and Collie. Median body weight was 19.7 kg (43.34 lb), and mean body weight was 18.4 kg (40.48 lb), with a range of 5.4 to 40.8 kg (11.88 to 89.76 lb). At the beginning of this study, all 38 (100%) dogs had signs of stranguria, 35 (92.1%) had macroscopic hematuria, 34 (89.3%) had pollakiuria, and 4 (10.5%) had straining while defecating.

Twenty-four of 38 (63.2%) dogs had not received prior treatment, and 14 of 38 (36.8%) dogs had received prior treatment. Of the 14 dogs, 2 underwent surgery, 9 received chemotherapy, and 3 received a combination of both. Of the 5 dogs undergoing surgery, none had clean surgical margins on histologic examination of the tumor. Twenty-six of 38 (68.4%) dogs were chemotherapy naive, while 12 of 38 (31.6%) dogs had received previous chemotherapy, consisting of 1 to 4 cycles of doxorubicin and cyclophosphamide. Three of these 12 dogs also received 2 to 3 cycles of cisplatin after having failed to respond to the previous protocol.

At the beginning of the study, 26 of 38 (68.4%) dogs were staged T1N0M0. The remaining 12 were staged T2N1M0 (n = 7), T1N2M0 (2), T1N0M1 (1), T1N1M1 (1), and T2N1M1 (1). The most common Primary treatment site in the dog staged M1 was the inguinal body wall and the skin adjacent to the prepuce. Overall, 4 of 38 (10.5%) dogs had metastatic disease at the beginning of treatment with gemcitabine.

**Dosing scheme and toxic effects of gemcitabine**—The median number of gemcitabine doses administered per patient was 8 (mean, 11; range, 1 to 38). All dogs were treated at a gemcitabine dose of 800 mg/m². A total of 407 gemcitabine treatments were given. The most common adverse effects were gastrointestinal tract signs, occurring in 26 of 38 (68.4%) dogs. Ten of 38 (26.3%) dogs had grade 1 gastrointestinal tract signs, 11 of 38 (28.9%) dogs had grade 2, and 5 of 38 (13.2%) dogs had grade 3. There was no dogs that developed grade 4 gastrointestinal tract signs. Generally, the onset of emesis was within the first 24 to 36 hours after treatment, but dogs recovered within 24 to 72 hours. Prophylactic antiemetics were not routinely given, although the owners were advised to give aminopentamide hydrogen sulfate tablets if required. The 5 dogs that had grade 3 gastrointestinal tract signs were thereafter prophylactically treated with a single IV injection of ondansetron at a dose of 0.15 mg/kg (0.06 mg/lb). Subsequently, none of them had clinically relevant drug-related nausea and vomiting. Three of 5 dogs that had grade 3 gastrointestinal tract signs were previously treated with other chemotherapeutic drugs, specifically doxorubicin and cyclophosphamide (n = 1) and doxorubicin, cyclophosphamide, and cisplatin (2). Twelve of 38 (31.6%) dogs did not have any adverse effects on the gastrointestinal tract during the entire course of treatment.

Overall, adverse hematologic effects were minimal. Neutropenia (grade 1 to 3) was the most common
adverse effect, which developed in 10 of 38 (26.3%) dogs, for a total of 12 neutropenic episodes during the study period. Grade 1 neutropenia developed in 6 dogs, whereas grade 2 neutropenia was observed in 2 dogs. Severe nonfebrile neutropenia (grade 3) occurred in 2 dogs, 3 days after the first treatment with gemcitabine in both dogs. Both dogs recovered without sequelae and had no need for hospitalization after IV administration of broad-spectrum antimicrobials.

A substantial degree of thrombocytopenia (grade 3) was detected in 2 dogs. Anemia was not observed in response to any dose of gemcitabine. None of the dogs that had myelosuppression developed febrile sepsis. Twenty-eight of 38 (73.7%) dogs did not have any adverse hematologic effects during the entire course of chemotherapy.

Further adverse effects, other than hematologic and gastrointestinal, were not observed. Overall, treatment delay or dose reduction because of persistent toxicity of gemcitabine in dogs was rare. The dose of gemcitabine was reduced in 11 patients because of adverse gastrointestinal or hematologic effects, whereas in 6 dogs, the treatment interval was increased to 10 to 14 days as a result of persistent neutropenia in 2 dogs and adverse gastrointestinal tract signs in 4 dogs. Body weight had no effect on adverse bone marrow (odds ratio, 1.03; 95% confidence interval, 0.93 to 1.14; $P = 0.55$) or gastrointestinal tract signs (odds ratio, 1.00; 95% confidence interval, 0.92 to 1.09; $P = 0.98$).

**Antitumor response**—With regard to antitumor activity, the responses of 37 dogs were evaluated. One dog was excluded from analysis because of it was lost to follow-up. Two of 37 (5.4%) dogs had a complete response, and 8 of 37 (21.6%) dogs had a partial response. The median number of gemcitabine treatments needed to achieve remission in these dogs was 7 (range, 2 to 10). Six of the 10 dogs with responses to gemcitabine treatment were still alive at the time of analysis, with a median remission time calculated to the study end of 159 days (range, 31 to 1,959 days). Nineteen of 37 (51.4%) dogs had stable disease, and 8 of 37 (21.6%) dogs had progressive disease. All 38 dogs had clinical improvement on the basis of reduced or subsided signs of stranguria, pollakiuria, and hematuria, within 1 to 2 cycles of gemcitabine treatment.

Two dogs achieved a complete response. One of them was a castrated male Bull Terrier that was staged T1N0M0 at the beginning of the study and had not been previously treated. This dog received 10 courses of weekly gemcitabine before achieving a complete response. Once a complete response was obtained, the dog started the maintenance protocol, consisting of biweekly gemcitabine treatment. This dog maintained its complete response for 575 days. The second dog achieving a complete response was a castrated mixed-breed dog staged T1N0M0 at the beginning of the study and being treatment naive at that time. This dog received 8 gemcitabine treatments before achieving a complete response. Once a complete response was obtained, only piroxicam was administered because of the owner’s financial constraints. At the time of writing (and 1,959 days after starting treatment), the dog was alive and still had a complete response.

Eight dogs achieved a partial response; none of them had been previously treated. All but 1 dog had T1N0M0 stage disease at the beginning of the study; the last dog was classified as T1N0Mx. Among these 8 dogs, 4 were alive at the end of the study, 3 had died because of their disease, and 1 dog was lost to follow-up. For the dogs that achieved a partial response to the treatment, it lasted for a median of 125 days (mean, 132 days; range, 31 to 274 days). Before observing a partial response, a median of 7 gemcitabine treatments had to be given (mean, 6; range, 2 to 9).

Eight dogs had progressive disease, and all of them had been euthanized because of their disease without attempting any rescue treatment. These dogs had advanced disease at the beginning of the study, as documented by TNM classification (4 dogs had T1N0M0, 1 had T1N0M1, 1 had T1N1M0, 1 had T1N3M0, and 1 had T2N1M0). Interestingly, 7 of these 8 dogs had received prior treatment before starting gemcitabine: 1 underwent surgery, 4 received chemotherapy, and 2 were treated with both. On the contrary, only 7 of the 29 (24.1%) dogs with stable disease, partial response, or complete response had received previous chemotherapy or surgery prior to starting the study. The frequency of previous treatment was significantly ($P = 0.002$) higher in dogs with progressive disease than those with other responses.

Most dogs ($n = 19$) had stable disease, and all of them had improvement in their clinical signs of disease with no tumor progression. Of the 19 dogs with stable disease, 6 had been previously treated, whereas the remaining were treatment naive.

At the beginning of the study, 4 of 38 (10.5%) dogs had metastatic disease, and during the study period, 3 additional dogs developed metastases to the iliac lymph node ($n = 1$), peritoneum ($n = 1$), and sacral vertebrae ($n = 1$); time to metastases in these dogs was 31, 80, and 699 days, respectively. At data analysis closure, 23 dogs had died, 12 were alive, and 3 were lost to follow-up. The cause of death was tumor related in all dogs.

Based on Kaplan-Meier analysis, median survival time for the 35 dogs was 230 days (95% confidence interval, 157 to 303 days) after gemcitabine was started (Figure 1). A univariate analysis of prognostic factors...
for overall survival time was performed (Table 1). Variables such as age, involvement of the urinary bladder or urinary bladder plus urethra or prostate, prior treatment, number of gemcitabine treatments, and type of response to gemcitabine had values of \( P < 0.15 \) and were retained in the multivariate analysis. With the adopted model, none of the listed variables remained associated with survival time. However, when age and number of gemcitabine treatments were considered as continuous variables, instead of categorical, and the multivariate analysis was repeated, the latter remained of prognostic value. For each additional gemcitabine treatment, survival time increased by approximately 10% (odds ratio, 1.09; 95% confidence interval, 1.02 to 1.17; \( P = 0.009 \)).

### Discussion

Over the years, several attempts have been made to improve outcome for dogs with TCC of the urinary bladder; however, discouragingly, median survival times have generally remained below 12 months with all available chemotherapeutic agents.\(^4,14\)

This study sought to evaluate the activity of the regimen of piroxicam and gemcitabine in the treatment of macroscopic disease. Gemcitabine is among the most promising chemotherapeutics in the management of advanced TCC in humans.\(^13,17,36\) This premise in addition to the similarities between invasive TCC in dogs and humans\(^37\) provided a rationale for studying this drug in dogs. Additionally, given the demonstrated activity of piroxicam in urothelial cancer,\(^4\) an evaluation of this drug given in combination with gemcitabine was undertaken, aiming at evaluating possible cytotoxic synergy. By use of clinical and ultrasonographic responses as an endpoint in our study, on an intent-to-treat basis, 2 of 37 (5.4%) dogs obtained a complete response. An additional 8 of 37 (21.6%) dogs obtained a partial response, adding up to a total response rate of 27.0%. Median survival time was 230 days.

By comparison, responses have been seen in 18% of dogs treated with piroxicam only with a median survival time of 181 days.\(^5\) Over the last 15 years, a number of new agents and combination regimens have been tested in advanced urothelial cancer. Previous trials showed that if combined with cisplatin or carboplatin, piroxicam improved response rate and, to some extent, median survival time. In fact, cisplatin combined with piroxicam induced remission in 50% to 71% of dogs with TCC, thereby showing the best promise in tumor control.\(^7,10\) More recently, carboplatin and piroxicam resulted in a partial remission rate of 40%.\(^16\) Nevertheless, response duration was short, and adverse gastrointestinal and hematologic effects were common. Taken together, median survival time in these trials ranged from 161 to 329 days.\(^7,10,18\)

### Table 1

Table 1—Results of Kaplan-Meier analysis of prognostic factors in 35 dogs that had TCC of the urinary bladder and received gemcitabine in combination with piroxicam treatment.

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>No. of dogs</th>
<th>Median survival time (d)</th>
<th>Hazard ratio ( (95% \text{ CI}) )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 10 y</td>
<td>14</td>
<td>180</td>
<td>1.89 (0.84–5.48)</td>
<td>0.11</td>
</tr>
<tr>
<td>Age ( \geq 10 ) y</td>
<td>21</td>
<td>241</td>
<td>0.66 (0.27–1.49)</td>
<td>0.30</td>
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<tr>
<td>Sex Male</td>
<td>18</td>
<td>252</td>
<td>1.43 (0.60–3.49)</td>
<td>0.40</td>
</tr>
<tr>
<td>Sex Female</td>
<td>17</td>
<td>188</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breed category</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purebred dog</td>
<td>23</td>
<td>220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed-breed dog</td>
<td>12</td>
<td>276</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight &lt; 10 kg</td>
<td>9</td>
<td>230</td>
<td>0.89 (0.34–2.35)</td>
<td>0.81</td>
</tr>
<tr>
<td>Body weight ( \geq 10 ) kg</td>
<td>26</td>
<td>220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNM stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 or T2</td>
<td>27</td>
<td>230</td>
<td>0.56 (0.17–1.40)</td>
<td>0.19</td>
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<tr>
<td>T2</td>
<td>8</td>
<td>180</td>
<td>0.43 (0.15–0.94)</td>
<td>0.04</td>
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<tr>
<td>Involvement</td>
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<td></td>
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<tr>
<td>Urinary bladder</td>
<td>23</td>
<td>252</td>
<td>0.35 (0.11–0.71)</td>
<td>0.01</td>
</tr>
<tr>
<td>Urinary bladder plus urethra or prostate</td>
<td>12</td>
<td>158</td>
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<tr>
<td>Prior treatment</td>
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<td></td>
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<tr>
<td>No</td>
<td>21</td>
<td>276</td>
<td>2.48 (1.04–5.64)</td>
<td>0.04</td>
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<tr>
<td>Yes</td>
<td>14</td>
<td>140</td>
<td>0.59 (0.20–1.46)</td>
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<tr>
<td>No. of gemcitabine treatments &lt; 10</td>
<td>21</td>
<td>149</td>
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<tr>
<td>No. of gemcitabine treatments ( \geq 10 )</td>
<td>14</td>
<td>616</td>
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<tr>
<td>Gemcitabine dose reduction No</td>
<td>22</td>
<td>252</td>
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<tr>
<td>Yes</td>
<td>11</td>
<td>180</td>
<td>0.23 (0.12–0.71)</td>
<td>0.01</td>
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<tr>
<td>Type of response to treatment</td>
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<td>Partial response or complete response</td>
<td>9</td>
<td>699</td>
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<td>Stable disease or progressive disease</td>
<td>26</td>
<td>162</td>
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<tr>
<td>No</td>
<td>25</td>
<td>241</td>
<td>0.70 (0.25–1.79)</td>
<td>0.43</td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>160</td>
<td>1.16 (0.46–3.04)</td>
<td>0.74</td>
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<tr>
<td>Adverse gastrointestinal tract signs No</td>
<td>12</td>
<td>220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>230</td>
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</table>

CI = Confidence interval.
Although a direct comparison was not made, by consideration of the published literature, it appears that the combination of piroxicam and gemcitabine has an overall response rate that approaches but does not surpass that of the platinum-based regimens, suggesting that the combination of piroxicam and gemcitabine is modestly active in the treatment of dogs with TCC of the urinary bladder. One possible reason for the low overall response rate might be the relatively poor prognostic group of dogs included in the present study. Indeed, 8 of 38 (21.1%) dogs had T1, stage disease at the beginning of the study, and 4 of 38 (10.5%) dogs had metastatic disease. During the study period, 3 additional dogs developed metastases. Notably, despite the reported metastatic rate of 40%, 1 metastases were not suspected in any other dog on the basis of clinical signs and ultrasonographic and radiographic findings; thus, it may be possible that gemcitabine suppressed metastatic growth in treated dogs. However, because necropsy was not routinely performed, micrometastatic disease may have been missed in some dogs. In addition to TCC of the urinary bladder, 7 of 38 (18.4%) dogs had also urethral involvement. In accordance with a finding in a previous study, urethral involvement decreased survival time in dogs of our study.

Another reason for the low response rate might be the result of patient heterogeneity with respect to previous treatment. Fourteen of 38 (36.8%) dogs received prior treatment, consisting of surgery, chemotherapy, or a combination of both, and were considered to have refractory or recurrent disease when the combined treatment with gemcitabine and piroxicam was first started. Interestingly, 7 of 8 dogs that had disease progression had received prior treatment before starting the gemcitabine and piroxicam combination, probably accounting for the lack of response. Previous chemotherapy in these dogs may have contributed to a pleiotropic drug resistance phenotype or to the alteration of the tumor microenvironment.

The low response rate may have been also caused by a nonoptimal treatment schedule or gemcitabine dose, which has not been established. Research in the human field indicates that gemcitabine trials should be designed on the basis of a schedule rather than a dose dependence because the time interval between chemotherapy administrations seems to be crucial when intermittent schedules are used. Here, pharmacokinetic analysis was not performed, thereby precluding assessment as to whether a different treatment schedule or higher dose would have improved outcome. The optimal number of gemcitabine treatments to administer is also unknown. The finding that, for each additional gemcitabine treatment, survival time increased by approximately 10% is likely to be incidental and should be interpreted with caution. In fact, dogs that survived longer are obviously those that also received more gemcitabine treatments.

Last, it is possible that ultrasonographic evaluation of T may have not been reliable for evaluating therapeutic response, although measurement standardization has been attempted. It is well known that, unlike other diagnostic techniques, such as computed tomography and magnetic resonance imaging, ultrasonography is rather subjective and examiner dependent, especially for urinary bladder lesions, where volumetric distortion is critical. Advances in imaging technologies allowing for volumetric tumor burden quantification as well as new tumor measurement techniques will possibly result in a different response assessment, compared with those of the 1-D and 2-D techniques. The observed median survival time was in the 8-month range, which overlaps a large number of other combination regimens. Here, 20% of dogs survived longer than 1 year, and this equals the results obtained with piroxicam administration alone. Unfortunately, despite the introduction of several cytotoxic agents with single-agent activity, efforts to dose intensify, and the use of combined regimens, little additional progress in improving outcome appears to have been made.

Regardless of the responses obtained, all treated dogs had an improvement of their clinical signs by the completion of 1 to 2 cycles, and none of the owners asked to withdraw from the study. Quality-of-life issues are important in veterinary medicine, and thus the major advantages of combined gemcitabine and piroxicam treatment are its decrease in adverse effects and improvement in clinical signs of disease. Indeed, in the present study, the combination was well tolerated. Drug-related toxicities were limited and in most cases readily amenable to supportive care. Treatment delays for unresolved toxic effects of gemcitabine occurred rarely. No signs of acute reaction were noted in any dog during drug delivery or immediately after.

In the present study, the most common adverse effect was gastrointestinal tract signs, with no patient having more than a grade 3 gastrointestinal tract sign. Five of 38 (13.2%) dogs had grade 3 gastrointestinal tract signs, whereas the most dogs (21/38 [55.2%]) had grade 1 to 2 self-limiting gastrointestinal tract signs. However, because enrolled dogs received piroxicam as well, it was difficult to determine whether adverse gastrointestinal tract signs were related to gemcitabine, piroxicam, or both. Concurrent piroxicam treatment may have enhanced nausea and vomiting. Of note, 31.6% (12/38) of dogs had no adverse gastrointestinal tract signs.

Neutropenia was the most common adverse hematologic effect, which developed in 26.3% (10/38) of treated dogs. Of the 31 dogs that received ≥ 4 gemcitabine treatments, 24 did not have any clinically relevant decrease in total WBC count, Hct, or platelet count over time. Therefore, we assumed that neutropenia was not cumulative, suggesting that gemcitabine does not irreversibly damage immature hematopoietic cells. In the present study, only 2 episodes of severe neutropenia that required aggressive treatment were reported. These dogs developed grade 3 neutropenia and thrombocytopenia 3 days after the first gemcitabine treatment. Both dogs recovered uneventfully and were treated at a 25% decreased dose thereafter with no further adverse effects. Thrombocytopenia was uncommon, whereas anemia was not observed in any of the treated dogs. There were no grade 4 hematologic effects, and no febrile sequelae occurred. Surprisingly, 73.7% (28/38) of dogs had no evidence of adverse hematologic effects. It can be speculated that prophylactic administration of antimicrobials PO prevented febrile episodes, thereby resulting in improved quality of life.

Previous trials exploring a combined cisplatin and piroxicam chemotherapeutic regimen found that ad-
verse renal effects were dose limiting in some of the treated dogs. 7,8 Adverse renal effects did not occur in our study, suggesting that the combined piroxicam and gemcitabine protocol may be better tolerated than the combined cisplatin and piroxicam protocol with regard to kidney function. Additionally, because dogs with advanced urothelial cancer may have impairment in renal function because of age, comorbid conditions, or disease-related factors limiting the use of platinum-based regimens, the piroxicam and gemcitabine combination may represent an effective alternative.

References