

TOLERABILITY AND TUMOR RESPONSE OF A NOVEL LOW-DOSE PALLIATIVE RADIATION THERAPY PROTOCOL IN DOGS WITH TRANSITIONAL CELL CARCINOMA OF THE BLADDER AND URETHRA

KEVIN CHOY, JANEAN FIDEL

Previously reported radiation protocols for transitional cell carcinoma of the canine lower urinary tract have been ineffective or associated with increased side effects. Objectives of this retrospective, cross-sectional study were to describe safety of and tumor responses for a novel palliative radiation protocol for transitional cell carcinoma in dogs. Included dogs had cytologically or histologically confirmed transitional cell carcinoma of the bladder or urethra, and were treated with 10 once-daily fractions (Monday–Friday) of 2.7 Gy. Thirteen dogs were sampled, with six treated using radiation as first-line (induction) therapy and seven treated using radiation as rescue therapy after failing previous chemotherapy. Within 6 weeks of radiation, 7.6% (1/13) dogs had a complete response, 53.8% (7/13) partial response, 38.5% (5/13) stable disease, and none had progressive disease. Three patients presenting with urethral obstruction had spontaneous micturition restored during the treatment protocol. A single patient with unilateral ureteral obstruction was patent at recheck examination. Median survival time from time of initial diagnosis was 179 days. Median survival time from start of radiation was 150 days. Acute radiation side effects occurred in 31% (4/13) patients and were classified as grade 1 or 2. No significant late side radiation side effects were reported. No variables examined were identified as prognostic factors. Findings indicated that the reported radiation protocol was safe in this sample of dogs with bladder and urethral transitional cell carcinoma. Future prospective studies are needed to determine utility of this treatment as a rescue therapy in patients with complete urinary tract obstruction. © 2016 American College of Veterinary Radiology.

Key words: dog, radiation, transitional cell carcinoma.

Introduction

URINARY BLADDER NEOPLASIA accounts for 2% of all canine cancers with transitional cell carcinoma the most commonly reported tumor of the lower urinary tract in dogs.^{1–4} Canine transitional cell carcinoma is most analogous to high-grade invasive transitional cell carcinoma in humans characterized by aggressive local involvement and moderate risk of metastasis.⁵ At time of diagnosis, 78% of dogs exhibit evidence of bladder wall invasion and 14%–20% have nodal or distant metastasis.^{3,5–8} Most are located in the trigone region of the bladder or trigone and urethra, which typically limits therapeutic options such as aggressive surgical resection. Many dogs with life-threatening urinary tract obstruction at time of diagnosis are euthanized or die from complications resulting from obstructive renal failure if untreated.⁴

Chemotherapy is currently the standard of care for management of transitional cell carcinoma in dogs. Cisplatin, carboplatin, doxorubicin, mitoxantrone, mitomycin C, and vinblastine-based chemotherapy protocols have been reported with measurable tumor response rates of between 0% and 38% and median survival times between 4 and 11 months.^{7–14} Use of Non-steroidal anti-inflammatory drugs (NSAIDs) including piroxicam, deracoxib, and firocoxib have been shown to have clinical efficacy against transitional cell carcinoma thought to be principally due to COX-2 isoform inhibition overexpressed in majority of canine TCCs with possible anti-inflammatory, immunomodulatory, and antiangiogenic effects.^{15–20} Cisplatin combined with piroxicam was also associated with an unacceptably high risk of renal toxicity.⁵ Mitoxantrone combined with piroxicam is the most commonly utilized protocol for transitional cell carcinoma resulting in a response rate of

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obstructive disease present at time of diagnosis, cytoreduction of tumor with other modalities has also been trialed.

Radical surgical resection via partial or subtotal cystectomy with urinary diversion techniques has been reported for bladder and urethral transitional cell carcinoma, particularly to relieve lower urinary tract obstruction but has been associated with poor quality of life, and short survival times of <5 months.²¹ En-bloc resection of the trigone and proximal urethra has also been described in a dog but resulted in serious complications including bladder necrosis and incontinence.²² Palliative techniques such as permanent cystostomy catheter, urethral stenting, transurethral resection, cystoscopic CO₂ laser, or ultrasound-guided endoscopic diode-laser debulking are also options but can be associated with significant morbidity and postoperative complications requiring additional treatments.²³⁻²⁷

Radiation therapy (RT), both intraoperative and external beam have been described for transitional cell carcinoma in limited studies. Intraoperative RT has been described in two studies with 1- and 2- year survival rates at 69% and 23%, respectively, with single intraoperative doses ranging from 10 to 35 Gy.^{28,29} High incidence (up to 56%) of severe late complications was noted with use of high fraction size radiotherapy including chronic colitis, cystitis, colonic/urethral stricture, and colonic perforation. Experimental radiation, however, of a 5-cm field of normal canine bladder trigone area at doses of 20-40 Gy, caused no early or late toxicity other than the development of a rhabdomyosarcoma within the treatment field in a single dog receiving 30 Gy.³⁰ Once weekly coarse-fraction radiation, mitoxantrone and piroxicam was evaluated in ten dogs and resulted in measurable tumor response in two dogs and median survival time (MST) of 326 days; comparable to chemotherapy alone. Late side effects occurred in four dogs consisting of cutaneous hyperpigmentation in a dog and urinary incontinence in three.³¹ A recent study utilizing intensity modulated radiotherapy (IMRT) in 21 dogs reported an overall median survival time of 654 days. No objective tumor response assessment was obtained but owner questionnaire-based subjective clinical response rate of 60% was achieved, with four dogs exhibiting late side effects of grade 3 gastrointestinal and genitourinary toxicity relating to stricture formation requiring surgical intervention (urethral stent, ureteral transposition, rectal stent).³² In vitro studies on transitional cell carcinoma cell lines examining dose response and sublethal radiation-induced damage repair exhibited a low-to-moderate α/β ratio suggesting that radiation protocols utilizing higher doses with less fractions may be more effective for treating transitional cell carcinoma.³³ However, this may increase complications seen with RT of the genitourinary tract and associated

abdominal structures. In general, radiation-associated urogenital toxicities reported include urinary incontinence, cystitis, bladder fibrosis, ureteral fibrosis with subsequent lower urinary tract structure and obstruction. Gastrointestinal toxicities reported include fecal incontinence, chronic colitis, fibrosis, stricture, and colonic perforation. To minimize the likelihood of these late side effects associated to the pelvic region, it has been suggested that a radiation protocol utilizing a dose per fraction of less than 3 Gy may be warranted.^{28,30,34-36} These observations were the basis for the initial choice of a low-dose per fraction in the study radiation protocol. A low total dose was chosen to limit acute effects, costs, and time in therapy; a low fraction size was chosen to limit late effects as the intention was to repeat the entire protocol at the relapse of clinical signs with the hypothesis that overall survival (OS) could be increased over use of chemotherapy alone and dogs could survive long enough that late effects could be seen.

The study was designed to be a proof-of-concept prospective study evaluating efficacy of a RT protocol as means of rapid induction therapy for bladder and urethral transitional cell carcinoma followed by previously described mitoxantrone and piroxicam chemotherapy. Enrolment initially was for patients with newly diagnosed transitional cell carcinoma of the bladder/urethra that presented to WSU Oncology service to receive this novel low-dose radiation protocol prior to start of standard chemotherapy with the goals of ameliorating tumor-associated lower urinary tract signs and increasing OS. However during the course of patient enrollment, more than half of our patients ($n = 7$) presented having previously failed medical management with chemotherapy and NSAIDs seeking other treatment modalities. This led to modification of our study to include evaluation of the radiation protocol as a potential rescue therapy as well with focus on palliative tumor response. The primary goal of this study was to describe tumor response and tolerability of a novel low-dose fractionated external beam radiation protocol in a group of dogs with transitional cell carcinoma of the bladder or urethra as a palliative treatment modality. Secondary objectives were to evaluate OS when this protocol was used as a first-line therapy (first-line RT) followed by chemotherapy, or as a rescue therapy (rescue RT) in chemotherapy-resistant patients. Our hypothesis was that the protocol would be effective in reducing tumor volume within the recheck period, and well tolerated in the early and late period as a palliative first-line or rescue therapy in conjunction with traditional systemic chemotherapy. We also hypothesized that OS would be longer than with historical medical management protocols where chemotherapy was the sole treatment modality.

Material and Methods

Patient Selection

The study design was retrospective, descriptive, and cross-sectional. Medical records of dogs that were treated with RT for documented transitional cell carcinoma of the bladder or urethra that presented to Washington State University Veterinary Teaching Hospital (WSU-VTH) oncology department between May 2009 and April 2013 were reviewed. Dogs with primary prostatic tumors (including prostatic carcinoma and suspected transitional cell carcinoma of the prostate only) were excluded. Complete local and systemic staging was required including a complete blood count, serum biochemistry profile, thoracic radiographs, abdominal ultrasound, abdominal computed tomography (CT), and if required, ultrasound-guided fine needle aspirate cytology of primary or metastatic lesions such as lymph node or organs. Staging diagnostics were performed within 2 weeks of planned RT. Planning CT was obtained in all study patients ($n = 13$) within 24–48 hours prior to starting RT. Abdominal CT imaging was performed with a Toshiba Aquilion 16-slice helical CT scanner, (Toshiba, Otawara-shi, Tochigi-ken, Japan) with 3 mm slices. Patients were positioned in lateral recumbency placed in a moldable positioning cushion (Vac-Lok, Civo Medical, Orange City, IA) and all received Ioxilan 300 at a dose of 1.5 ml/kg (Oxilan 300, Guebert LLC, Bloomington, IN) for contrast enhancement. Adjuvant or neoadjuvant chemotherapy was not permitted within 1 week before start of RT until recheck imaging 3–6 weeks after treatment. Concurrent chemotherapy and radiation during the study period was not allowed. Nonsteroidal anti-inflammatory drugs were permitted throughout the treatment period; before, during, and after RT.

One observer (K.C.) recorded the following data from each patient's medical records: signalment, history pertaining to lower urinary tract signs, TNM stage,¹ previous treatment timing of radiation and chemotherapy, response to treatment, local tumor control, RT toxicity, and OS. When necessary, patient data recording was completed through follow-up communication with primary care veterinarians, referring oncologists and pet owners. All patients were followed until death or until the end of the study period.

Radiation Therapy Protocol

All included dogs were treated with megavoltage radiation delivered with a linear accelerator (Elekta SL15, Philips Medical Systems North America, Bothell, WA) using 6 or 15 MV photons. All patients received ten fractions of 2.7 Gy totaling 27 Gy given once daily (Monday to Friday) over 12 days. All dogs were anesthetized via mask induction with subsequent endotracheal intubation for maintenance of general anesthesia with des-

flurane and oxygen. Patients were treated in lateral recumbency placed in a moldable positioning cushion (Vac-Lok, Civo Medical, Orange City, IA). Port films were used daily to establish position and to roughly visualize the size of the bladder to be sure the field was treating the tumor site. Digital films (Fujifilm type IP cassette, Fuji Photofilm Co. LTD, Tokyo, Japan) were used for larger field size and greater tissue discrimination than onboard imaging allowed, and were reviewed by one of the authors (J.F.). The gross tumor volume (GTV) was defined as any mass and/or areas that were contrast enhancing on the CT. The clinical tumor volume (CTV) was defined as a minimum of a 1-cm margin of normal tissue around the GTV if in both bladder and urethra, and incorporated the entire bladder or urethra if only one of the two was involved. A margin of 0.5 cm was added for the planning treatment volume (PTV). Two, parallel-opposed lateral beams were used. Local draining lymph nodes, if cytologically positive for metastasis, were also treated either as a separate field or incorporated into the field using a PTV of 0.5-cm margins around the nodal GTV field. The dose was planned to the 95% isodose line with the entire GTV receiving at minimum 100% of the dose and the CTV receiving at minimum 90% of the dose. No heterogeneity correction was made. Organs at risk (excluding the urinary tract) included spinal cord and colon. No calculated dose (in computer-assisted plans) was allowed in the spinal cord but maximum dose to colon/rectum was not restricted as long as the dose was given to less than 50% of the circumference of the organ. If urethral blockage was present, an indwelling Foley catheter was placed to establish urinary patency before CT imaging to be incorporated into the treatment planning and remained in place until evidence of spontaneous micturition detected via leakage around the catheter was observed.

Tumor Response and Toxicity Assessment

Response to therapy was evaluated utilizing repeat imaging within 3–6 weeks after treatment. All patients initially had abdominal computed tomography (CT) prior to therapy. Repeat lower urinary tract imaging was all evaluated by board-certified veterinary radiologists. Lower urinary tract obstruction was categorized into either urethral or ureteral obstruction. Urethral obstructions were based on inability to micturate spontaneously or with manual expression, requiring urethral catheterization. Ureteral obstruction was based on CT findings consistent with unilateral hydroneurter, hydronephrosis, and lack of urethral contrast column connecting the affected kidney to the bladder lumen. All measurements were compared against the same imaging modality utilized at initial baseline evaluation (ultrasound examination for all patients except for one, that had double-contrast cystography before and after radiotherapy) imaging was repeated immediately following

spontaneous micturition to minimize the effect of bladder filling on measurement variability in an attempt to further standardize measurement conditions. For localized lesions, tumor size was determined using modified response evaluation criteria in solid tumors (RECIST) guidelines.^{37,38} Following complete abdominal ultrasound examination with orthogonal imaging of the urogenital tumor, the longest diameter of the tumor was recorded, if more than one lesion was present, the largest tumor diameter was used in both pre and postradiation abdominal ultrasound images and compared together to determine tumor response. In the single patient utilizing double-contrast cystography, reports and images from a board-certified veterinary radiologist were utilized pre and postradiation therapy. Complete response (CR) was defined as when tumor was no longer detectable, partial response (PR) was defined as a greater than 30% decrease in longest dimension, SD if less than 30% decrease or less than 20% of tumor growth and progressive disease (PD) if greater than 20% increase in longest dimension was observed. For patients that attained a cytologic diagnosis of urogenital carcinoma via percutaneous sampling, any description of tumor involving the caudal abdominal peritoneal surface, musculature or associated subcutaneous tissues in the region of the needle tract on subsequent recheck imaging during patient follow-up was considered evidence for needle-tract implantation or seeding of tumor.

Radiation toxicity was assessed through review of the daily patient record maintained during RT and patient history as reported by the client and physical examination findings at subsequent visits. Acute and late radiation effects were assessed and scored according to the toxicity criteria of the Veterinary Radiation Therapy Oncology Group (VROG).³⁹

Statistical Analysis

Statistical analyses were conducted by one author (K.C.) using a commercially available statistical software program (MedCalc 12.4 Statistical Software, Ostend, Belgium). Radiation survival (RS) was defined as time (in days) from first radiation treatment to time of death. Overall survival was defined as the time (in days) from initial cytologic or histologic diagnosis to time of death. Patients were censored if dogs were alive at the end of the study, or died from disease unrelated to transitional cell carcinoma. Disease-free interval was not evaluated due to non-uniform follow-up timing and imaging modalities after the 6-week recheck period at WSU. Survival was generated using Kaplan-Meier product-limit method and compared via log-rank test with a *P*-value of ≤ 0.05 considered significant. Variables analyzed for prognostic significance were response within 6 weeks, tumor site (bladder vs. urethra), age (above or below 10 years), sex, body weight, local or distant metas-

tasis at time of diagnosis, biopsy method utilized (cytology vs. histopathology), urinary tract blockage at time of presentation, and timing of RT in relation to chemotherapy (first-line RT vs. rescue RT).

Results

Patient Data

Thirteen dogs met study inclusion criteria and all presented to the medical oncology service at WSU (Appendix 1). One author (K.C.) was primary clinician of nine of the 13 cases during the study period. Nine dogs were spayed females, two intact females and two were neutered males. Median age of dogs was 11 years (range, 4–13 years), median weight was 25.5 kg (range, 5.9–37.4 kg). Ten distinct breeds were represented including Labrador retriever ($n = 3$), Golden retriever ($n = 2$) and one of each of the following breeds: Border collie, West Highland terrier, Jack Russell terrier, Miniature schnauzer, Rough coated collie, Australian shepherd, Australian cattle dog, and Chow mixed-breed dog. Tissue diagnosis was obtained for histopathology in six dogs via a variety of biopsy techniques including cystoscopy ($n = 3$) and open cystotomy ($n = 3$). Cytologic diagnosis of urogenital carcinoma was obtained in nine dogs via percutaneous ultrasound guided fine needle aspirate ($n = 6$) and traumatic catheterization ($n = 3$). Two dogs had both cytologic and histologic diagnosis. None of the dogs that underwent percutaneous sampling had evidence of needle tract tumor implantation and no negative impact was noted on OS during the follow-up period. Clinical signs consistent with lower urinary tract disease including hematuria, urinary incontinence, pollakiuria, stranguria, and urinary tract obstruction were present from 1 week to 9 months before diagnosis with a median of 3.5 months. Lower urinary tract obstruction was the principal clinical sign at time of diagnosis in four dogs (three urethral, one ureteral).

Tumor Staging and Previous Therapy

Six dogs were treated with RT as first-line therapy (first-line RT) prior to planned four doses of mitoxantrone chemotherapy with concurrent NSAID under the supervision of the medical oncology department. Seven dogs were referred for radiation after failing a variety of therapies and a variety of therapies were used after RT (rescue RT). Eight dogs had localized disease ($T_{2,3}N_0M_0$), two had regional lymph node metastasis ($T_{2,3}N_1M_0$) and three had evidence of distant metastasis ($T_{2,3}N_{0,1}M_1$) to lung ($n = 2$) and liver ($n = 1$) at time of first RT. Tumors were located principally in the bladder in ten dogs, with nine in the trigone and one in the cranial apex. All dogs had gross disease. Primary urethral carcinoma was identified in three dogs, all of

which were female ($n = 1$) or spayed ($n = 2$). Four patients had evidence of lower urinary tract obstruction at time of RT. Six dogs were treated with RT as first-line therapy prior to four doses of mitoxantrone chemotherapy with concurrent NSAID. Two of these were obstructed (both $T_1N_0M_0$ with urethral and ureteral obstruction one each). Seven dogs were treated with RT (rescue RT) after documented progressive disease while on chemotherapy and two of these presented with urethral obstruction ($T_1N_0M_0$ and $T_1N_1M_0$, respectively). Prior chemotherapy treatments included single agent piroxicam ($n = 6$), mitoxantrone ($n = 3$) and individual patients treated with firocoxib, carboplatin, doxorubicin, vincristine, vinblastine, and toceranib alone or in combination. Five dogs had failed more than two medical treatments prior to RT. For patients that initially exhibited clinical benefit from initial radiation (first-line therapy) but then subsequently had progressive disease on adjuvant mitoxantrone therapy, other therapeutic modalities were offered including, carboplatin, satraplatin, vinblastine, lomustine, and alternate NSAIDs including deracoxib and firocoxib. Salvage techniques for end-stage urethral obstruction were used in two dogs including laser diode ablation and permanent percutaneous low-profile cystostomy catheter.

Radiation Therapy

Seven dogs had hand calculated plans with the treatment field incorporating the entire bladder when partially distended, or entire urethra, as identified on a radiograph or CT scan image. The dose was calculated to the center of the dog using parallel opposed lateral beams. Computer-assisted planning was utilized in six patients (Prowess, 1844 Clayton Road, Concord, CA 94520) with all plans calculated on noncontrast enhanced images.

Response and Survival Assessment

All 13 dogs completed their prescribed RT. All patients were reexamined within 3–6 weeks after completion of RT with continued follow-up until death.

Median time between diagnosis and start of RT for all dogs was 17 days (1–215 days). Repeat imaging was available for all 13 dogs in the study within the 6-week period. Recheck imaging modalities included computed tomography ($n = 3$), abdominal ultrasound ($n = 9$), and double-contrast cystography ($n = 1$) and were all reviewed by board-certified radiologists. A single dog had CR, seven dogs had PR, and five dogs had SD resulting in an overall response rate of 61.5% (8/13). No patients had evidence of progressive disease based on imaging. Subjective clinical improvement in lower urinary tract signs was seen in 10 of 13 dogs within the initial recheck period based on decreased reported pollakiuria and/or stranguria. Extent

and duration of this amelioration of clinical signs could not be ascertained from the medical records. Four patients had evidence of complete lower urinary tract obstruction at time of diagnosis. All three of the urethral obstructions were patent following two, five, and seven fractions of radiation at three, six, and eight days, respectively, with spontaneous micturition without urethral catheterization. One patient with unilateral ureteral blockage and associated hydroureter was patent on ultrasound examination at 6 weeks following completion of RT.

Using Kaplan–Meier survival curve analysis the median OS for all patients was 179 days (range, 50–767 days; Fig. 1) with a mean of 265 days. When categorized by first-line vs. rescue RT, median OS of dogs first-line RT group was 156 days (range, 75–767 days) vs. the rescue RT group with 270 days (range, 50–450 days; Fig. 2). Log-rank analysis indicated that timing of radiation either before or after chemotherapy was not significantly ($P = 0.8847$) associated with OS time. A single patient was censored from analysis due to death from nontransitional cell carcinoma related cause from metastatic grade III mast cell tumor.

Median RS time from first treatment for all patients was 150 days (range, 25–763 days; Fig. 3) with a mean of 221 days. When categorized by first-line vs. rescue RT, median treatment survival time of dogs with first-line RT was 150 days (range, 74–763 days) vs. rescue RT with 147 days (range, 25–365 days; Fig. 4). Log-rank analysis indicated that timing of radiation either before or after chemotherapy was not significantly ($P = 0.5160$) associated with treatment survival time. None of the variables analyzed as potential prognostic markers were significantly associated with survival.

Side Effects

Acute side effects were noted in four patients that were mild to moderate including one each: self-limiting grade 1 perineal dermatitis, grade 2 colitis, grade 2 cystitis, grade 2 vaginitis; all of which resolved within the 6-week recheck period. No patients developed grade 3 toxicity. Conservative medical management for grade 2 cystitis and vaginitis (in addition to continuing NSAID therapy) consisted of additional analgesia such as tramadol for 1–2 weeks following onset of clinical signs. Grade 2 colitis was managed with a 5-day course of tylosin (10–15 mg/kg PO q12 h) and fiber supplementation. Tylosin was selected due to onset of clinical signs of tenesmus and mucoid hematochezia consistent with large intestinal diarrhea and concerns of potential radiosensitizing activity of nitroimidazole-class drugs such as metronidazole for gastrointestinal tissues within the treatment field.⁴⁰

No clinically significant late RT side effects were noted but leukotrichia was noted in two dogs in the shape of the parallel opposed fields within the treatment field on the

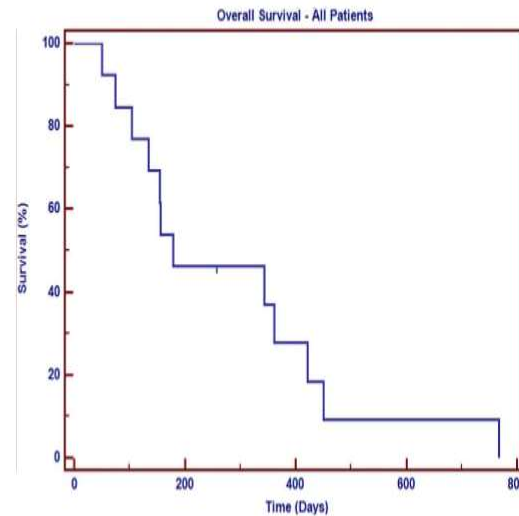


FIG. 1. Kaplan-Meier survival curve for dogs treated with palliative radiation therapy (RT) pre or postchemotherapy. The overall median survival time was 179 days.

lateral abdomen. Seven of 13 patients were followed greater than 6 months from therapy with a median follow up time of 361 days (range, 179–797 days). None of the variables analyzed as potential prognostic markers were significantly associated with development of side effects.

Discussion

Signalment data for the dogs in this study were consistent with previous reports except for a higher female to male ratio of 11:2 (previously reported ratio of 1.71–1.91:1^{3,4}) and a higher proportion of gundog retriever breeds ($n = 5$) that may reflect regional differences in breed populations within North America. A measurable tumor response was observed with our radiation protocols demonstrated by an overall response rate of 61.5% within the 3- to 6-week follow-up recheck period. Of clinical interest was the ability of our radiation protocol to rapidly restore luminal patency in patients that presented with complete lower urinary tract blockage. All three dogs with urethral obstructions were able to micturate without urethral catheter within two to seven fractions of radiation over a period of 3–8 days. A single case of unilateral ureteral obstruction with renal pelvis dilation and mild hydronephrosis was also resolved at 6-week recheck ultrasound examination following RT.

The median and mean OS time in the present study was relatively modest at 179 and 265 days, respectively.⁷ Though direct comparisons between studies cannot be made, studies utilizing medical management alone such as mitoxantrone combined with piroxicam resulted in relatively longer median survival time of 350 days.¹⁰ The reasons for this observed decrease in OS is unclear but may be due, in part to potential case selection bias as patients more severely affected with lower urinary tract signs and higher stage disease may have been more likely to elect for first-line RT. All dogs that received RT as first-line (induction) therapy followed by chemotherapy were recruited through the WSU medical oncology service. Additionally, approximately half of accrued dogs were offered RT in a rescue-salvage type setting following failure of traditional chemotherapy. The inclusion of chemotherapy-resistant patients may also have further contributed to selection bias for more aggressive tumor phenotype that negatively impacted OS.

The median and mean treatment survival time from the first day of RT was 150 and 221 days, respectively. This measure was evaluated to reduce confounding factors on OS such as prior treatments with chemotherapy. When categorized further by RT use as first-line therapy vs. rescue therapy; median treatment survivals from first treatment to death were 150 and 147 days, respectively, which was not statistically different ($P = 0.5160$). This suggested that prior

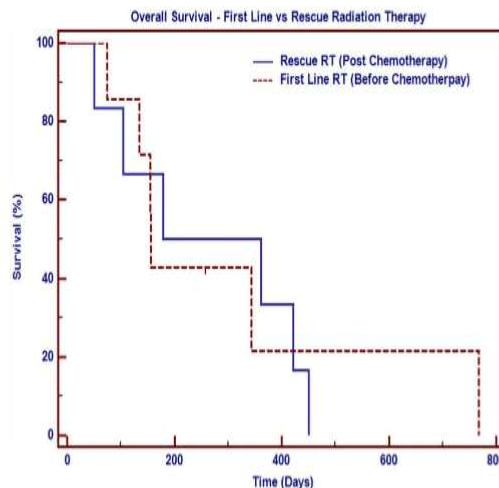


FIG. 2. Kaplan-Meier for dogs treated with palliative radiation therapy (RT) categorized as first-line vs. rescue RT. The median overall survival time for first-line RT was 156 days vs. rescue RT of 270 days. Log-rank analysis was not statistically significant ($P = 0.8847$).

chemotherapy did not significantly impact tumor response of the described palliative radiation protocol, as patients that underwent radiation in the presence of chemotherapy-resistant disease were able to receive additional clinical benefit in extended survival of approximately 4 months. All patients clinically benefited from this low-dose palliative radiation protocol, particularly those that presented with complete or severe lower urinary obstruction that otherwise would not have survived long enough to clinically benefit from traditional medical management with chemotherapy either as induction therapy or when transitioning to alternate cytotoxic drug regimens.

Radiation therapy for high-grade transitional cell carcinoma in humans is typically utilized as part of multimodal therapy including surgery and chemotherapy where complete excision is not feasible either due to extensive local invasion or metastasis. The optimal total dose and dose per fraction in humans for bladder carcinomas is not yet determined.⁴¹ Similarly, there has been considerable variability in radiation protocols used for canine transitional cell carcinoma. Radiation sensitivity in tumors can in part be extrapolated from review of α/β ratio values in experimental tumors in *in vitro* models, though their utility in guiding clinical RT planning is considered controversial. Recently, a study examining dose response and sublethal radiation-induced damage repair of canine transitional cell

carcinoma cell lines *in vitro* revealed a low-to-moderate α/β ratio (3.27), suggesting that radiation protocols utilizing higher doses per fraction with less fractionation may be more effective for treating transitional cell carcinoma.³⁵ However, previous studies evaluating the use of coarse fractionated RT using intraoperative radiation, particularly fields that involve the trigone or ureter suggest that a high total dose may increase risk of complications associated with radiotherapy of the genitourinary tract. Increased incidence of ureteral fibrosis and stenosis was observed in this study when average total RT dose exceeded 30 Gy.²⁸ Subsequent studies of late complications involving irradiation of pelvic tumors in dogs, reduced occurrence of side effects by reduction of fraction size to less than 3 Gy (2.7–2.9 Gy).³⁴

The biologically effective dose (BED) is a model used to compare different radiotherapy fractionation schemes and their effect on the early and late responding tissues. With this calculation, for the early responding tissues (using $\alpha/\beta = 10$) 10×2.7 Gy compared with a standard protocol of 30×2 Gy, delivers 34 Gy₁₀ vs. 72 Gy₁₀. A slightly higher dose is delivered to the late responding tissues (using $\alpha/\beta = 3$) at 51 Gy₃ for our protocol vs. 100 Gy₃ for a standard protocol. This was considered very tolerable in both the early and the late period and was developed to give what was considered a safe dose when repeated in the event of clinical relapse. If repeated, a total of 20×2.7 Gy would

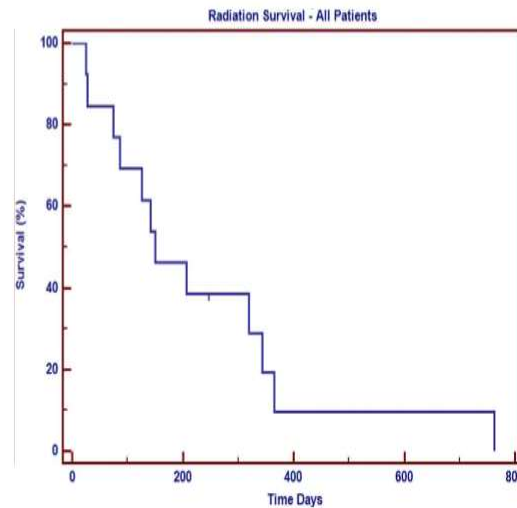


FIG. 3. Kaplan-Meier survival curve for dogs treated with palliative radiation from time of first radiation therapy (RT) treatment. The radiation median survival time was 150 days.

be delivered; the early tissue BED would be 68.58 Gy₁₀ and the late would be 102.6 Gy₅. Of the dogs that developed radiation associated toxicity, most were grade 1 and 2 and localized within the treatment field. Clinically significant long-term side effects were not noted within the study period with leukotrichia only noted in three dogs in the shape of the parallel opposed fields on each side of the lateral abdomen. Overall the RT protocol was well tolerated.

Limitations of this study include small sample size, retrospective nature, and lack of standardization of therapy in relation to radiation timing, chemotherapy protocols used before and after RT. Though identification of prognostic factors was not an objective of this study, none of the variables evaluated were found to be statistically significant. These included sex, body weight, age, urethral involvement, obstruction at presentation, metastasis, biopsy method utilized, timing between initial diagnoses to RT, timing of radiation in relation to chemotherapy, and development of radiation related toxicoses. It is likely that this is due to the small sample population of which to compare subsets against one another, increasing the possibility of a type II error. This reflects the general difficulty in obtaining patient enrolment and data in the veterinary field, particularly in uncommon cancers that further emphasize the need for centralized clinical trials in veterinary oncology. A 1-week washout restriction was imposed on

patients receiving chemotherapy prior to start of palliative RT to minimize the confounding effect on objective response. The authors recognize that the role of NSAIDs that were allowed throughout the treatment period and potential prolonged drug action of cytotoxic chemotherapy agents such as mitoxantrone and carboplatin that are typically given on a 21-day cycle cannot be fully ruled out. The rationale for the 1-week washout period was to not only screen for side effects such as immunosuppression nadir following chemotherapy, but as more than half (7/13) of the study population presented with progressive disease in the face of prior chemotherapy, with four patients with complete lower urinary tract obstruction, a full 3-week washout period or exclusion of NSAID-class drugs was not clinically advisable in most patients. Multiple imaging modalities utilized to assess response to therapy, which was performed by different board-certified radiologists with a 3- to 6-week range following RT. Bladder tumor assessment via pre- and post-radiation computed tomographic imaging with standardized bladder filling for volumetric tumor assessment and interpretation by the same radiologist at strict intervals following therapy would have been ideal, it was not practically feasible for majority of our patients due to financial and geographical limitations. To minimize variability, board-certified radiologist reports were utilized in all cases and imaging was performed immediately following

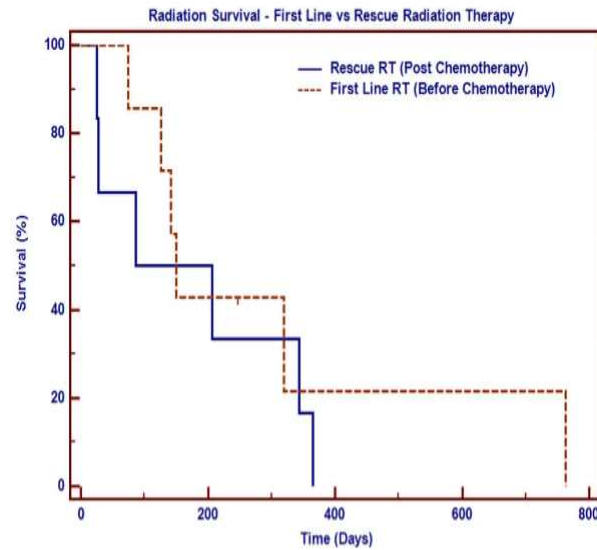


FIG. 4. Kaplan-Meier for dogs treated with palliative radiation therapy (RT) categorized as first-line vs. rescue RT. The median overall survival time for first-line RT was 150 days vs. rescue RT of 147 days. Log-rank analysis was not statistically significant ($P = 0.5160$).

spontaneous micturition to reduce the effect of artifactual changes induced by bladder filling.

In conclusion, the results of the present study demonstrated that the novel palliative low-dose radiation protocol described as first-line or rescue therapy was well tolerated, with minimal radiation associated toxicity in both the early and late phase for this sample of dogs. Measureable antitumor effects in urogenital carcinoma of the bladder and urethra were observed, with a 61.5% overall response rate in this study population. Radiation therapy was able to restore urinary patency in dogs with urethral and ureteral obstruction and may present an additional treatment modality for the management of transitional cell carcinoma of the bladder and urethra in dogs. Future stud-

ies are needed to determine whether this low-dose palliative radiation protocol may be useful to downstage local disease burden prior to start of chemotherapy in patients with advanced stage disease or as a palliative rescue modality in the face of chemotherapy-resistant disease. Further controlled clinical trials for the protocol, particularly as a rescue therapy with concurrent cytotoxic chemotherapy, are also needed.

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APPENDIX I. Summary of Patient Data

| PT | Breed | Age (Years) | Sex | Tumor location | TNM stage | Prior treatment | Post treatment | RECIST response | Days to unblock | RT toxicity | RTS (days) | OS (days) | Cause of death |
|----|-----------------------|-------------|-----|--|---------------------|---|---|-----------------|-------------------------------|---------------------------------|------------|-----------|--|
| 1 | West Highland Terrier | 8 | MC | Trigone, medial iliac LN | T3N1M0 (LN) | Carboplatin Doxorubicin Piroxicam | Metronomic lomustine | PR | - | - | 207 | 422 | Local Progression |
| 2 | Golden Retriever | 11 | F | Trigone, urethra, vagina | T3N0M0 | - | Carboplatin Mitoxantrone | PR | 3 (2 RT fractions) | Perineal dermatitis (Grade I) | 126 | 134 | Local Progression |
| 3 | Australian Shepherd | 12 | FS | Urethra | T3N0M0 | Mitoxantrone Piroxicam | None | PR | 8 (7 RT fractions) | - | 25 | 179 | Distant Metastasis (Liver, Lung) |
| 4 | Labrador | 12 | FS | Apex | T3N0M0 | - | Mitoxantrone | PR | - | Colitis (Grade II) | 248 | 258 | Grade III Mast Cell Tumor |
| 5 | Border Collie | 12 | FS | Distal urethra, vagina | T3N0M1 (Lung) | - | Mitoxantrone | SD | - | Vaginitis/Urethritis (Grade II) | 150 | 156 | Local Progression |
| 6 | Rough Coated Collie | 4 | MC | Trigone, majority of bladder lumen | T3N1M1 (Lung + LN) | Mitoxantrone Piroxicam Chlorambucil Vinblastine Toceranib | Toceranib Metronomic Lomustine | SD | - | - | 28 | 50 | Distant Metastasis (Brain) |
| 7 | Labrador Retriever | 10 | F | Urethra | T3N0M0 | - | Mitoxantrone | SD | - | - | 74 | 75 | Local Progression |
| 8 | Miniature Schnauzer | 9 | FS | Trigone, urethra | T3N0M0 | Piroxicam | Mitoxantrone | SD | - | - | 87 | 104 | Distant Metastasis (Spinal, Cutaneous) |
| 9 | Australian Cattle Dog | 13 | FS | Trigone, urethra | T3N0M0 | - | Mitoxantrone Carboplatin | SD | - | - | 320 | 343 | Distant Metastasis (Lung) |
| 10 | Chow Mix | 12 | FS | Trigone, R hydronephr, R hydronephrosis (mild/partial) | T3N1M1 (Liver + LN) | Mitoxantrone Piroxicam Vincristine | Carboplatin - 5FU Metronomic Satraplatin Laser Diode Ablation | PR | - | - | 365 | 450 | Anesthetic Complications During Laser Ablation |
| 11 | Jack Russell | 10 | FS | Trigone | T3N0M0 | Piroxicam | Mitoxantrone | CR | - | - | 763 | 767 | Distant Metastasis (Liver) |
| 12 | Golden Retriever | 9 | FS | Trigone, L hydronephrosis | T3N0M0 | - | Mitoxantrone Toceranib | PR | Hydronephrosis Resolved at 6w | Cystitis (Grade II) | 142 | 155 | Local Progression |
| 13 | Labrador Retriever | 11 | FS | Trigone, urethra, medial iliac LN | T3N1M0 (LN) | Piroxicam Firocoxib | Mitoxantrone Vinblastine Carboplatin Metronomic Chlorambucil | PR | 6 (5 RT fractions) | - | 343 | 361 | Local Progression |

Abbreviations: MC = Male castrated, F = Female intact, FS = Female spayed, LN = lymph node, PR = partial response, SD = stable disease, CR = complete response, RT = radiation therapy.

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