# A combined protocol with piroxicam, chemotherapy, and whole pelvic irradiation with simultaneous boost volumetric modulated arc radiotherapy for muscle-invasive canine urinary transitional cell carcinoma: First clinical experience

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**ABSTRACT.** The aims of this pilot study were to evaluate the feasibility and efficacy of high-dose hypofractionated volumetric modulated arc radiotherapy (VMAT) applied to whole pelvic region radiotherapy (WPRT) with multilevel simultaneous integrated boost (MLSIB) combined with *piroxicam* and chemotherapy for the treatment of canine transitional cell carcinoma (TCC) of the lower urinary tract with muscle invasion TCC. Twelve dogs were enrolled, according to stage, in two groups: group 1, TCC confined to the urinary tract; group 2, TCC with metastasis. The planning target volume dose was tailored from 36 to 42 Gy in 6 fractions. All dogs were prescribed piroxicam and radiosensitizing carboplatin, and six received chemotherapy after radiotherapy. Serial follow-ups with computed tomography and magnetic resonance imaging were performed. Disease control and toxicity effects were evaluated according to the Response Evaluation Criteria in Solid Tumors and Veterinary Radiation Therapy Oncology Group criteria. The treatment was well tolerated, and no high-grade side effects were reported. The median overall survival times for groups 1 and 2 were 1,230 and 150 days, respectively. A considerable percentage of patients in group1 (50%) were still alive at the time of writing this paper, and a longer follow-up could enable a more accurate survival analysis. This preliminary analysis shows that VMAT applied to the WPRT with MLSIB is an effective and safe option for dogs with lower urinary TCC, although the presence of metastases worsens the prognosis.

**KEYWORDS:** chemotherapy, dog, piroxicam, radiotherapy, transitional cell carcinoma

Transitional cell carcinoma (TCC) is the most common cancer of the canine urinary tract, accounting for <2% of canine malignant tumors [26, 34–36, 54, 57]. According to the World Health Organization (WHO) criteria for staging canine bladder tumors [60], 78% of dogs with TCC have been reported to have T2 tumors (invading the bladder wall, i.e., muscle-invasive), and 20% have T3 tumors (invading neighboring organs) [26, 36, 61]; papillary lesions and a thickened bladder wall are frequent features that can lead to partial or complete urinary tract obstruction [75]. In male canines, the prostate gland may also be involved [41].

Dogs with invasive TCC have different treatment options, including *cyclooxygenase* (COX) inhibitors (e.g., piroxicam, deracoxib, and pirocoxib) [26, 32, 34–36, 38, 39, 57, 63], cytotoxic chemotherapy drugs (cisplatin, carboplatin, mitoxantrone, vinblastine, gemcitabine) [1, 19, 26, 28, 34–36, 39, 54], metronomic chemotherapy (chlorambucil) [13, 20, 23, 24, 47–49, 64, 68, 69], laser ablation [14, 26, 43], urethral or ureteral stent placement [9, 26, 52, 74], surgery [11, 26, 29, 34, 35, 50, 54, 66, 67, 70, 76, 77], and other radiation therapy protocols [55, 56, 62, 73]; however, a single treatment that leads to a robust response is lacking.

Piroxicam is a single agent that is generally well tolerated in palliative treatment [38], and lifelong use is recommended, even with complete remission [63]. Treatment is discontinued if progressive disease or unacceptable toxicity is noted.

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Because of the typical trigonal location and the urethral involvement of the tumor, complete surgical excision of the TCC is not usually possible [63]. Moreover, many dogs develop multifocal TCC in the bladder. In patients where the tumor is located at the apex of the bladder, partial cystectomy is an option [29, 71]. Additionally, surgery can be used as a palliative procedure for debulking inoperable tumors in dogs with distal urinary tract obstruction [14].

Despite the high rate of metastasis in canine TCC, progression of local disease is the cause of death for many dogs in which the primary tumor is not adequately controlled [35], demonstrating the need for a combined approach for both local and systemic treatments.

The standard radiotherapy (RT) for lower urinary tract malignancies in humans is whole pelvic region radiotherapy (WPRT) [12, 15, 25]. The rationale for this treatment is to limit the progression of the disease to the lymphatics and neighboring organs. Studies on RT in canine TCC using conventional techniques are limited because of the difficulty in treating many targets simultaneously [2–4, 6, 10, 12, 14, 15, 18, 25–27, 34, 36–39, 42, 48, 51, 53, 56, 60, 62, 71, 73], and no extensive studies have been devoted to WPRT. Using the 3D conformal conventional techniques of RT for the lower urinary tract TCC poses a challenge in dose escalation trials and often results in induced sub-optimal outcomes as TCC is often locally advanced at the time of diagnosis. Moreover, daily variation in the position and dimension of the abdominal organs, especially for the bladder, suggests an increase in target margins in the planning target volume (PTV) definition with unwanted dosages to the surrounding healthy organs, toxicity, and unexpected results [3–5]. Complications of normal pelvic tissue irradiation with 3D conformal radiotherapy and the difficulty of targeting the treatment volumes are the major limiting factors for treating canine TCC patients with hypofractionated radiation [3, 25, 48]. The side effects reported in the literature for conformal 3D radiotherapy for TCC treatments include pollakiuria, urinary incontinence, cystitis, stranguria, and hydronephrosis [3, 12, 15, 25, 48, 53]. Additionally, a tumor near the urethral orifice may result in bladder outlet obstruction and urinary retention. Other infrequent clinical signs include lameness caused by bone metastasis [16].

Based on these considerations, this pilot study was performed to evaluate the feasibility and effectiveness of a combined treatment with piroxicam and chemoradiotherapy for overcoming the technical difficulties of standard 3D conformal radiotherapy through volumetric modulated arc radiotherapy (VMAT) in hypofractionated dose regimens and with multilevel simultaneous integrated boost (MLSIB) characterized by different dose levels for the whole pelvic region irradiation and tumor volumes.

## MATERIALS AND METHODS

The pilot study and the radiotherapy (RT) treatment protocol were approved by the local scientific ethics committee (date, November28, 2013; record number 07/2013). Dogs were recruited from 2013 to 2018, and the follow-up lasted until the time of writing the paper. All enrolled patients underwent a preliminary clinical examination and a complete blood count (CBC), serum biochemical profile, urinalysis, and ultrasound imaging [50] were performed in addition to histologic confirmation of the diagnosis of TCC/urothelial carcinoma.

Animals were anesthetized for diagnostic investigations, and midazolam (Istituto Biochimico Italiano Giovanni Lorenzini S.p.A., Aprilia, Italy) was used as premedication. All dogs underwent endotracheal intubation and were ventilated with Vetflurane isoflurane (Virbac Italia s.r.l., Milan, Italy) in mixed medical grade air and oxygen, with continuous monitoring of vital signs.

Based on imaging, the tumor was diagnosed and staged in agreement with the Clinical Staging System of the WHO [60].

Complete staging of the tumor was obtained with Brilliance 64 Slice computed tomography (CT) [58] (Philips Medical Systems, Amsterdam, the Netherlands) and/or Intera 1.5Tmagnetic resonance imaging (MRI) (Philips Medical Systems).

Total body CT scans were acquired before and after the injection of 300 mmol/ml contrast iodinated medium administered at a dose of 2 ml/kg iopamidol (Bracco, Milan, Italy). CT was used to evaluate the involvement of the urinary tract, to identify the involvement of the tributary or distal lymph nodes, and to detect the eventual presence of metastasis.

*Patients were grouped into two groups*: group 1 included dogs with tumors confined to the urinary tract; group 2 included dogs with lymph node involvement and/or metastasis. Any concomitant disease was registered.

In all dogs, therapy with piroxicam oral capsule [27, 31, 38, 62, 73] (Mepha Pharma AG, Aesch, Switzerland) at 0.3 mg/kg once a day was prescribed after the staging.

All dogs except one received carboplatin therapy during irradiation [17, 44, 46]. The basic requirements for chemotherapy were as follows: absence of comorbidity, adequate medullary reserve, and absence of renal dysfunction.

Carboplatin (Teva Pharmaceutical Industries, Petah Tikva, Israel) was administered at a dose of 25 mg/m$^2$ as a radiosensitizer [7, 12, 24] before each radiotherapy treatment. Carboplatin chemotherapy and cyclophosphamide (Baxter International Inc., Deerfield, IL, USA) metronomic chemotherapy [13, 20, 23, 24, 47, 48, 68, 69], as defined by the National Cancer Institute in its dictionary, at doses of 75 mg/m$^2$/week and 10 mg/m$^2$/day, respectively, were proposed to all dog owners after the end of the radiotherapy course, but only six people accepted it.

WPRT with MLSIB was performed. Volume treatment definitions included tumors with appropriate margins (PTV-tumor), lymphatic nodes (PTV-lymphatics), the entire bladder (PTV-bladder), the prostate and urethra (PTV-urethra), and the entire pelvis except the rectal volume (PTV-pelvis).

The PTV-tumor was determined by adding the volume of probable tissue invasion and the internal and setup margins to the tumor mass. The primary organs at risk (OAR) that were contoured were the colon and rectum, spinal cord and *cauda equina*, small bowel, and hip joints.

RT treatment was administered using a 6-MV photon beam with a SynergyS (Elekta Instrument AB, Stockholm, Sweden) linear accelerator (LINAC) equipped with a micromultileaf beam collimator (Elekta Beam Modulator) and an XVI cone-beam computed

tomography (CBCT) system. VMAT treatments were planned with the Elekta CMS Monaco treatment planning system (TPS) and a Monte Carlo statistical algorithm.

The internal protocol for the radiotherapy treatment was based on the following [21]:

• Use of a customized repositioning device made with a homemade cradle and an indexing system for the CT and LINAC couch. For all patients, preliminary CT scans were performed to evaluate the best treatment setup. In particular, to optimize the dose distribution, each dog was scanned in lateral, dorsal, and sternal recumbency to study the displacement of the OAR and the target. In the CT simulation, the bladder was manually emptied and subsequently filled with customized saline solution.

• Two-step virtual simulation. Three radiopaque markers were embedded in the cradle, roughly defining the target isocenter position (setup reference point) in the first virtual simulation step. Second, after the target was contoured, the real target isocenter was identified, and a set of shifts from the setup reference point was calculated for the correct LINAC positioning procedure.

Customized treatment plan elaboration with defined dose constraints for all patients

The PTV coverage was considered acceptable for $V_{95\%}$ and $V_{107\%}$ levels (PTV volume receiving less than 95% and more than 107% of the prescribed dose, respectively) of less than 5% and 1%. Accordingly, the heterogeneity index (HI), defined as the ratio between the dose delivered to the hottest 5% of the tissue ($D_5$) and the minimum dose received by 95% of the tissue ($D_{95}$), was considered satisfactory when less than 1.12. The OAR dose constraints were roughly derived from human studies (human percentages or total volume cannot simply be adapted), as described by the American Association of Physicists [8] in Medicine Task Group 101 because no specific dose constraints have been reported in the veterinary literature. Ultimately, constraints were iteratively lowered during the elaboration of the plan while preserving the target coverage unless otherwise specified by the veterinarian.

*Pretreatment feasibility analysis*

A per-patient agreement between the planned and delivered doses was performed before the beginning of each treatment using the Delta4 (Scandidos, Uppsala, Sweden) system and the gamma function [45] (dose agreement of 3% and a distance to agreement of 3 mm).

*Setup error evaluation*

Each treatment session began with a CBCT acquired through the Elekta XVI system. The obtained scan and the planning CT were compared, and the shifts were analyzed as previously described [21]. If agreement was not achieved, the whole treatment procedure was repeated starting from the CT simulation. All shifts were registered and analyzed to provide feedback on setup reproducibility and repositioning device effectiveness.

All patients were treated at approximately the same time of the day. Our preparation protocol was similar to that reported in the literature [56]. Briefly, meals of equal quantity were withheld 8 hr before each radiotherapy session, and defecation and urination were encouraged by walking prior to sedation. If the bladder size was significantly smaller at the pretreatment CBCT than the simulation CT, intravenous fluid therapy was used to restore the proper bladder volume. The filling difference was accepted within 2 mm along the major axis of the bladder. Because of variable patient and bladder size in relation to the target margin, there was no standard intravenous fluid bolus volume. In contrast, if the bladder size exceeded the amount planned, urethral catheterization or gentle manual expression of the urinary bladder was performed. In both cases, it was necessary to use CBCT to verify the correct positioning of the patient and the bladder dimensions after the operations.

The prescribed dose ranged from 36 to 42 Gy in six fractions, three times a week, depending on the body condition score and according to OAR proximity and dose tolerance. The equivalent total dose for fractions of 2 Gy (EQD2) was between 63.3 and 81.9 Gy using a tumor alpha/beta ratio of 3.27, as reported by Parfitt *et al.* [48].

Regular clinical examinations were performed during and after RT. Since dogs with TCC are at increased risk of contracting secondary bacterial infections, urinalysis and urine culture were performed periodically, while CBC and serum biochemical profiles were steadily evaluated in patients who received chemotherapy. Particular attention was devoted to the analysis of the quality of life in terms of return to urination in obstructed patients. Serial CT and MRI examinations were performed 2, 4, and 8 months after the end of RT and then according to clinical requests. The diagnostic imaging protocol for follow-up examinations consisted of repetition of one of the imaging techniques (CT or MRI), with the same scanning parameters used for the diagnostic exam and the same degree of bladder distension. Thickness, alterations in the organ wall, and neoplasm extension were adopted to evaluate the tumor variation and response according to the Response Evaluation Criteria in Solid Tumor (RECIST) [22] categorization and implemented with clinical follow-up examinations. Radiation toxicity was evaluated clinically and graded according to the Veterinary Radiation Therapy Oncology Group (VRTOG) [40] criteria.

Overall survival time (OST) and local progression-free survival (PFS) were estimated using the Kaplan–Meier [33] curve analysis method for the two patient groups and for all the patients kept together. All deaths and the progression of local disease were considered as events for OST and PFS, respectively. The median OST and 95% confidence interval (CI) were calculated considering the time to the event from the end of radiation therapy and the differences in OST and PFS between the two groups, and the whole set of data was statistically analyzed using a log-rank test.

## RESULTS

In total, 12dogs with lower urinary tract TCC were included in the study: 6 intact males, 1 neutered male, 3 intact females, and 2 spayed females, with a median age of 11 years (mean, 11 years; range, 8–13 years). Presenting complaints were typically hematuria

and pollakiuria; dogs with urethral obstruction had dysuria, while dogs with metastasis showed pelvic pain.

Breed stratification and main clinical information are reported in Table 1. There was heterogeneous localization of neoplasms. In two dogs, surgical biopsy was performed before irradiation due to apex and ventral wall localization.

According to the criteria from the WHO [60], eight dogs were classified as T2N0M0 (group 1), while four dogs were classified as T3N1M1 (group 2).

At the time of diagnostic CT, the median PTV-tumor was 55.1 cm$^3$ (range, 3.2–329.7 cm$^3$). The prescribed doses ranged from 36 to 42 Gy in all dogs, and the main plan elaboration data are summarized in Table 2. Almost all plans fulfilled the prescribed requests for both the target coverage and the OAR sparing since the median 95% isodose volume coverage ($V_{95\%}$) for the PTV-tumor was 96% (range 94.7–99.5%), the median 107% isodose volume excess coverage ($V_{107\%}$) was 0.1% (range 0–2.1%), the HI was 1.09 (range 1.07–1.11), and the median doses with their standard deviations for the colon and rectum were 11 Gy (range 1.2–29.4 Gy) and 23.8 Gy (range 7.4–34.9 Gy), respectively. In Figs. 1 and 2, the dose distribution and dose-volume histogram (DVH) are shown for a representative case. Additionally, the agreement between the planned and delivered doses resulted in a median value of 98% (range 95–99%) and was considered satisfactory, while the analysis on setup accuracy showed good reproducibility of our homemade repositioning device with similar results to that reported earlier [21].

**Table 1.**  Breed stratification and main clinical data

| Dog | Breed | Age (Years) | Body weight (kg) | Sex | Localization | Stage | Tumor Volume Pre-RT | Tumor response | Survival (Days) | Cause of death |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Crossbreed | 10 | 12 | M | Bladder | T2N0M0 | 57.40 | CR | 276 | Refused treatment (vertebral metastasis) |
| 2 | Crossbreed | 13 | 24 | M | Bladder | T2N0M0 | 55.10 | PR | 355 | Recurrence of tumor in bladder and lymphatics not treated |
| 3 | Crossbreed | 13 | 29 | F | Bladder and urethra | T2N0M0 | 64.90 | CR | 1,230 | Pulmonary disease |
| 4 | Crossbreed | 13 | 8 | M | Bladder | T3N1M1 | 82.67 | PR | 264 | Euthanized−vertebral infiltration and pulmonary mass |
| 5 | Crossbreed | 12 | 9 | SF | Bladder and urethra | T2N0M0 | 35.53 | No follow-up | 238 | Euthanized |
| 6 | Cocker Spaniel | 10 | 12 | SF | Bladder and urethra | T3N1M1 | 3.33 | PR | 209 | Urinary tenesmus− refused medical therapy |
| 7 | Setter | 8 | 22 | M | Urethra Prostate | T3N1M1 | 51.75 | SD | 151 | Difficulty in urination− refused medical therapy |
| 8 | Crossbreed | 8 | 32 | NM | Bladder, urethra, and prostate | T3N1M1 | 83.50 | PD | 36 | Euthanized |
| 9 | Toy Poodle | 13 | 11 | SF | Bladder | T2N0M0 | 14.02 | PR | | Alive |
| 10 | French Bulldog | 10 | 13 | M | Bladder | T2N0M0 | 77.73 | CR | | Alive |
| 11 | Border Collie | 11 | 22 | F | Bladder | T2N0M0 | 29.72 | PR | | Alive |
| 12 | Crossbreed | 12 | 12 | M | Bladder | T2N0M0 | 30.00 | CR | | Alive |

M, male; F, female; SF, sterilized female; NM, neutered male. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

**Table 2.**  Plan optimization results

| Dog | PTV volume (cc) | $V_{95\%}^{PTV}$ | $V_{107\%}^{PTV}$ | HI | Mean dose rectum (Gy) | Mean dose colon (Gy) |
|---|---|---|---|---|---|---|
| 1 | 88.5 | 95.0 | 0.1 | 1.09 | 23.9 | 22.2 |
| 2 | 55.1 | 95.2 | 0 | 1.07 | 31.3 | 38.2 |
| 3 | 65.2 | 95.9 | 0 | 1.08 | 31.7 | 28.7 |
| 4 | 36.7 | 96.0 | 0.1 | 1.09 | 34.9 | 37.5 |
| 5 | 6.7 | 99.0 | 0 | 1.07 | 8.6 | 35.4 |
| 6 | 77.9 | 96.4 | 0 | 1.09 | 7.4 | 22.7 |
| 7 | 29.7 | 94.7 | 0.3 | 1.10 | 18.4 | 36.7 |
| 8 | 30.0 | 95.2 | 0.1 | 1.11 | 10.4 | 40.6 |
| 9 | 82.2 | 96.8 | 2.1 | 1.11 | 31.5 | 20.4 |
| 10 | 3.2 | 99.5 | 0 | 1.07 | 30.7 | 34.5 |
| 11 | 51.8 | 95.0 | 0.1 | 1.10 | 23.6 | 37.7 |
| 12 | 329.7 | 97.2 | 0 | 1.07 | 8.1 | 33.3 |

PTV, planning target volume; $V_{95\%}^{PTV}$, planning target volume receiving the 95% of prescription dose; $V_{107\%}^{PTV}$ planning target volume receiving more than the 107% of prescription dose; HI, homogeneity index.
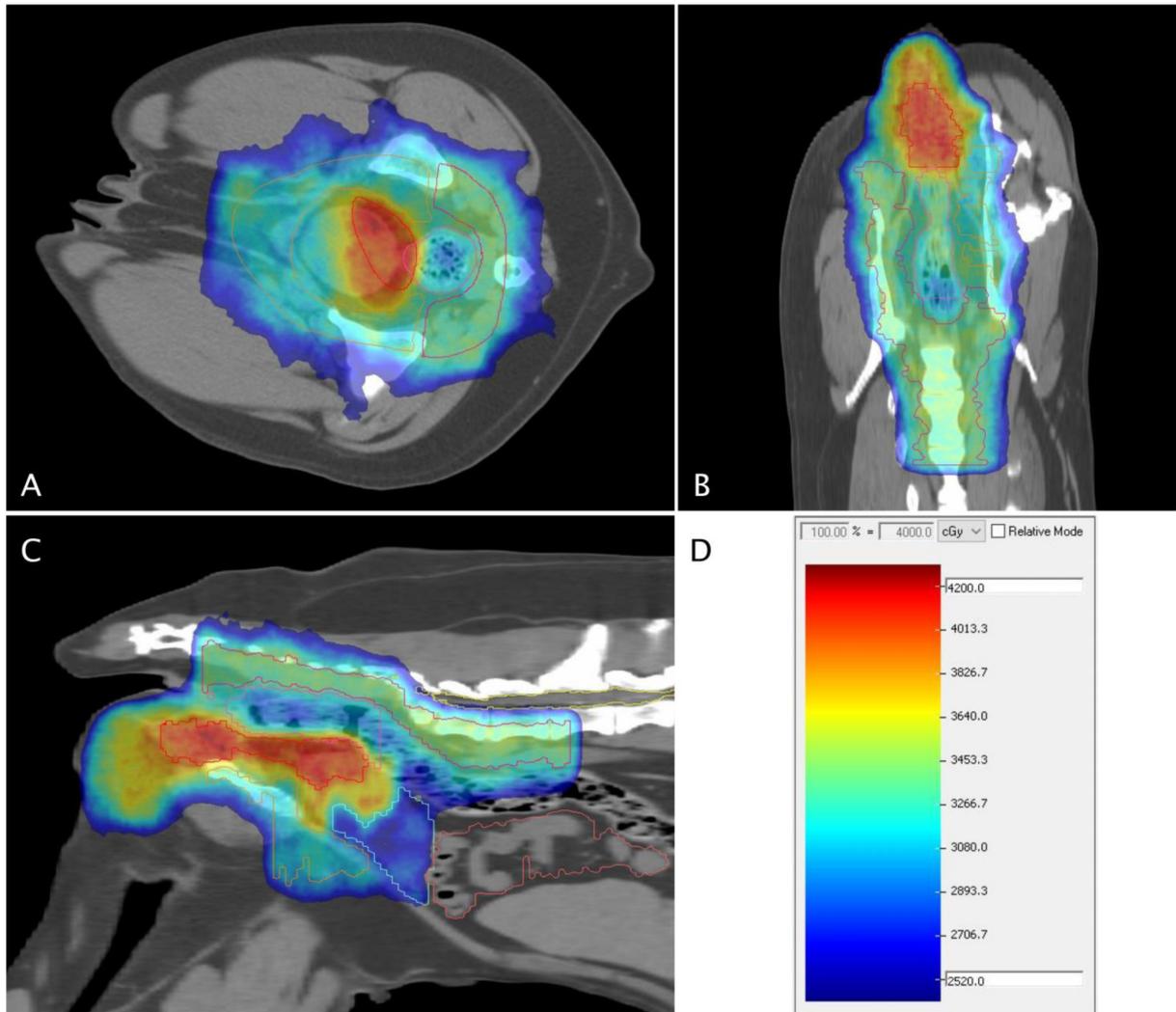
Fig. 1. Dose distribution of a representative case. (A) transversal projection; (B) coronal projection; (C) sagittal projection; (D) dose level distribution.
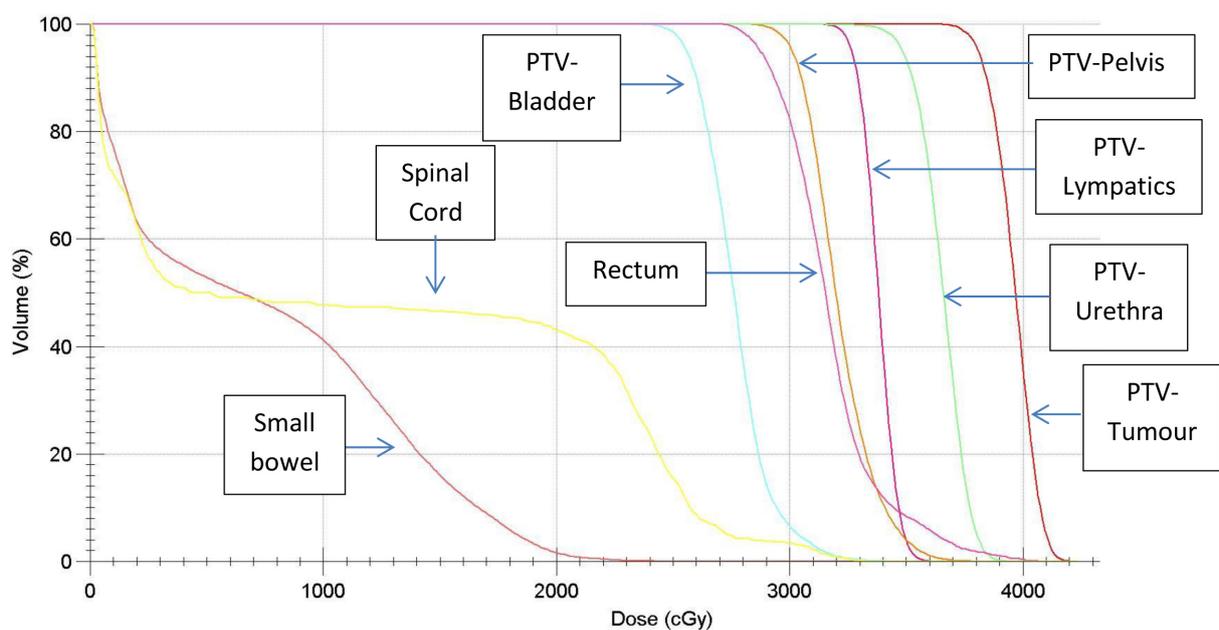


Fig. 2. Dose-volume histogram (DVH) of a representative case.

After radiation therapy, six dogs were treated with chemotherapy: four dogs with carboplatin weekly (75 mg/m/week) and two dogs with metronomic cyclophosphamide. All dogs showed progressive shrinkage of the tumor during follow-up, as shown in Fig. 3, where volumes are shown at the diagnosis and two months after the end of radiotherapy for a typical case. According to RECIST, four dogs had a complete response (CR), five dogs had a partial response (PR), one dog had stable disease (SD), and one had progressive disease (PD) (Table 1). One patient was lost at the first follow-up, and we learned about the patient's death in the subsequent interview with the owner. After transient low-grade cystitis (VRTOG 1), there were no signs of adverse radiation effects on MRI or clinical examination in most patients, and the initial complaints were regressed.

At the time of data analysis, eight deaths were recorded, while four dogs that were still alive were censored in the Kaplan–Meier OST analysis, as shown in Fig. 4, where the OST results are reported for each of the patient groups separately and as a combined group. The median OST for groups 1 and 2 were 1,230 and 210 days, with a 95% CI range of 1,230–1,230 and 116–302 days, respectively; the same data for the whole patient set included 360 days with a 95% CI range of 229–481 days. Since there were an equal number of events and censored data in group 1, the median and range values had limited clinical significance. For this reason, the mean results were also reported, showing values of $800 \pm 220$, $210 \pm 30$, and $630 \pm 170$ days for group 1, group 2, and all patients, respectively. Similarly, the ranges were 373–1,219, 144–272, and 298–961 days. The log-rank analysis showed a statistically significant difference ($P$=0.0035) in OST between groups 1 and 2, while no other pairwise multiple comparisons yielded significant differences. Similarly, the results for PFS are shown for each patient group separately and as a whole (Fig. 5). For groups 1 and 2, the median PFS was 780 and 160 days, respectively, while the value for the whole patient set was 220 days.

As for the OST, the mean PFS values were $500 \pm 140$, $150 \pm 18$, and $400 \pm 110$ days for group 1, group 2, and all the patients, respectively, while the 95% confidence interval ranges were 234–783, 119–188, and 187–611 days, respectively. The log-rank analysis showed no statistically significant difference in PFS between any pairwise multiple comparisons. The OST rates for group 1 and all dogs that were kept together were 54% and 38%, respectively, at one year while no dogs were still alive from group 2. Similarly, the reported rates for local PFS were 58% and 40%, respectively.

## DISCUSSION

To the best of our knowledge, no combined chemotherapy and RT studies have been conducted in veterinary medicine with WPRT and MLSIB. This strategy could be an important factor for limiting local tumor progression and may improve the outlook for progression or death due to local disease; however, it could also limit tumor spread remotely via lymphatics first to the lymph nodes and then to the lungs, liver, and bones through the blood stream. In our study, almost all the plans fulfilled the prescribed target dose request, and a good agreement between the prescribed and delivered doses was revealed, demonstrating the treatment feasibility. The use of a dedicated cradle with a vacuum-locked bag and the LINAC XVI CBCT check, performed before each session, enabled a reproducible patient setup.
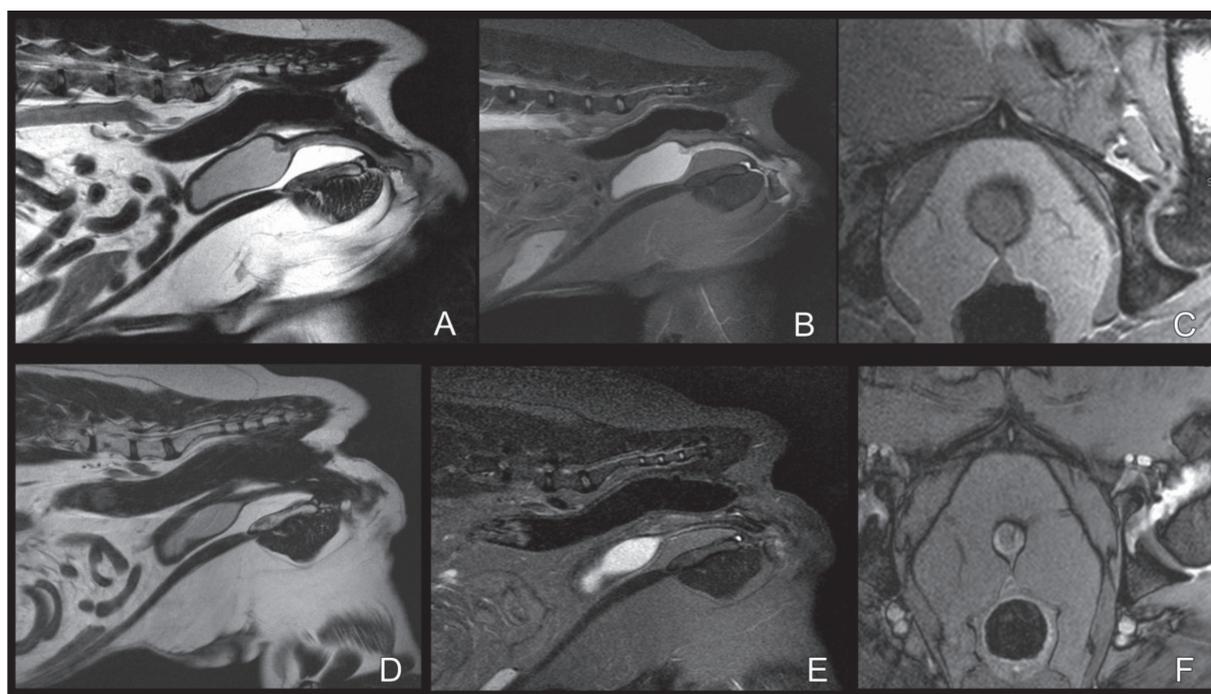


**Fig. 3.** Dog #3, Transitional cell carcinoma of the urethra, MRI of the caudal abdomen and pelvis (A and D, sagittal Turbo Spin Eco (TSE) T2-weighted; B and E, sagittal Short tau inversion recovery (STIR); C and F, axial contrast enhanced Fast Field Echo (FFE) T1-weighted at the level of the cranial urethra) at diagnosis (A, B, C) and two months after the end of the RT treatment (D, E, F), demonstrating complete response.
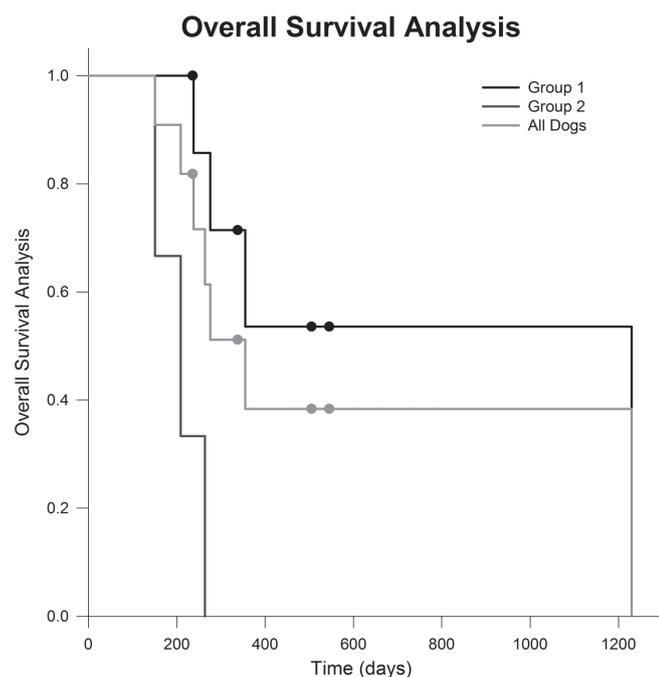
**Fig. 4.** Kaplan-Meier overall survival time analysis curve. In black, the 8 patients from group 1; in gray, the 4 patients from group 2; in light gray, all patients kept together.



**Fig. 5.** Kaplan-Meier progression free survival time analysis curve. In black, the 8 patients from group 1; in gray, the 4 patients from group 2; in light gray, all patients kept together.
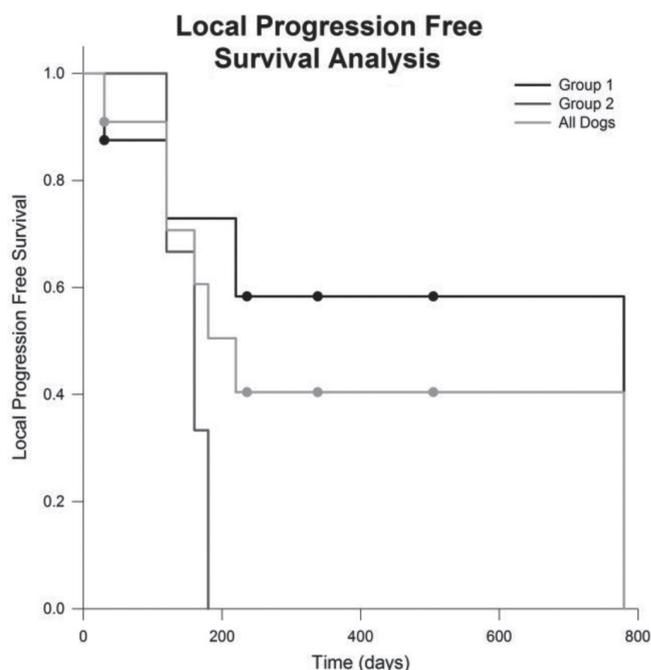
All dogs showed progressive shrinkage of the tumor during follow-up, and according to RECIST, four dogs had a CR, five dogs had a PR, one dog had SD, and one dog had PD. One patient was lost at the first follow-up, and the owner informed us of the animal's death at the subsequent interview.

A study of normal tissue complications in 51 dogs undergoing definitive pelvic region irradiation with $Co^{60}$ gamma ray standard conformal radiotherapy [5] reported acute side effects, such as epithelial desquamation and mucositis, and late complications, such as chronic colitis/enteritis, gastrointestinal perforation, rectal/anal fistulas, strictures (urinary, rectal, and gastrointestinal), myelopathies, and bone necrosis. In another study [73], high single doses administered intraoperatively caused severe late radiation toxicity, resulting in euthanasia in up to 36% of dogs with TCC. According to the two previously cited studies, a fraction size >3 Gy with standard conformal 3D RT was associated with a high risk of late effects [5, 73]. The dose prescription in our study was higher than the 3 Gy limit. Moreover, the WPRT with MLSIB was irradiated, but no clinical or radiological toxicity was observed. In particular, in some patients, VRTOG 1 level signs, such as pollakiuria, urinary incontinence, cystitis, and stranguria, with a duration of a few days were observed. No gastrointestinal complications were reported. Only one dog had proctitis with a duration of 3 days (VRTOG 1), and one dog presented with grade 1 late fibrosis.

In the study by Nolan *et al.* [56], 21 dogs with genitourinary carcinomas were irradiated with intensity-modulated and image-guided radiation therapy on the lymph nodes in addition to the primary tumor, but not in prophylactic treatment as in this study. In contrast, in our study, the rest of the bladder and/or urethra and the rest of the pelvis were irradiated in addition to the primary tumor and lymph nodes. The tumor dose in the study by Nolan *et al.* ranged from 54 to 58 Gy (while for the lymph nodes, doses ranged from 28 to 54 Gy) delivered in 20 daily fractions, corresponding to an EQD2 range of 61.2 to 63.22 Gy considering the alpha/beta ratio of 3.27 reported for this pathology in the literature [59]; this was similar to the lower limit of our dose prescription (63.3 to 81.9 Gy). The absence of severe side effects showed the safety of the protocol and could be related to the high conformity obtained using the VMAT technique, which permitted good target coverage while sparing the OAR, suggesting dose escalation trials with a possible improvement in tumor control probability (TCP).

Carboplatin (25 mg/m$^2$) was used in our study as a radiosensitizer. Only six dogs received chemotherapy, also after the end of the RT course. This could have provided a further advantage, but this effect is yet to be determined. However, it is not possible to provide suggestions about the component of the multimodal approach that provides the main contribution to PFS. Moreover, additional therapy could have improved long-term outcomes. A combined radio-chemotherapy protocol was presented in a previous study [62], where in piroxicam and mitoxantrone were administered in combination with once-weekly course fraction radiation therapy (six weekly fractions of 5.75 Gy) in 10 dogs. One dog experienced acute effects of radiation therapy (grade 1 dermatitis), and late side effects occurred in four dogs (cutaneous hyperpigmentation and urinary incontinence). Regarding survival, dogs in group 1 had a greater median OST (1,230 days) in comparison with the same value reported in the literature regarding various treatment modalities, but the high percentage of patients still alive at the time of writing this paper (50% of group 1 and 33% of the whole patient set) led us to consider the mean OST value (800 days for group 1) as a more descriptive parameter than the median

value, even if, with such a small sample, normal distribution cannot be confirmed and OST and PFS have small statistical meaning, having been evaluated mainly for a comparison with the reported literature. In this frame, by including the patients from group 2 with a pure palliative intent, the mean OST (600 days) result was comparable to that reported by other authors.

A recent study [15] reported that the median OST for canine TCC medically treated with COX inhibitors and/or systemic chemotherapy was 196 days. On multivariable analysis, TCC localization (hazard ratio [HR], 1.90; $P$=0.037), bone metastasis (HR, 2.76; $P$=0.013), and sternal lymphadenomegaly (HR, 3.56; $P$=0.004) were significantly associated with survival. Compared to the bladder localization, the urethral localization had higher metastasis rates to the bone (6.3% versus 42.3%; $P$=0.045) and lung (6.3% versus 46.2%; $P$=0.022). The survival time was shorter in the urethral localization than in the bladder localization (121.5 days versus 420 days; $P$<0.001). Other studies [2, 4, 10, 18, 27, 30, 31, 35, 37–39, 48, 51, 68] reported a median survival time ranging from 152 days to 323 days for COX inhibitors alone and from 147 days to 307 days for patients receiving a combination of chemotherapy and COX inhibitors.

In a study by Nolan *et al.* [56] based on intensity-modulated RT, a median OST of 654 days was reported, while in our study, the corresponding value was 1,230 days; moreover, 326 days were reported by Poirier [62] for a chemoradiotherapy combined treatment where the protocol was well tolerated, but the course fractionation therapy did not result in enhanced response rates compared to a previous study of dogs treated with mitoxantrone and piroxicam without radiotherapy [31]. Retrospective studies of a combination of chemotherapy protocols showed a median survival of 259 days with a combination of doxorubicin and cyclophosphamide treatment in 11 dogs [30] and 358 days with anthracycline and platinum drug combinations in 15 dogs [65]. Administration of cisplatin (60 mg/m$^2$) combined with piroxicam (0.3 mg/kg) produced the highest percentage of remission in dogs, but this combination caused renal toxicity, and a higher dose of cisplatin is not recommended [27]. Based on published data, the absolute survival benefit for chemotherapy-treated dogs was modest at best, strongly supporting the need for the development of more effective regimens.

Regarding surgery, Norris [57] reported a post-surgery median survival of 125 days in 23 dogs, and Helfand [30] reported 86 days in 14 dogs with TCC. However, most dogs with this disease are usually deemed unsuitable [31, 54] because of trigonal location or advanced tumor stage; only 2 dogs from our study qualified for surgical resection. Partial cystectomy was considered an option in dogs with apex or mid-body localization [51], with a PFS of 235 days and a median survival time of 348 days (772 days in dogs receiving daily piroxicam). Additionally, laser ablation combined with piroxicam and mitoxantrone was investigated [72], with median and mean progression-free intervals of 200 and 280 days, respectively, and median and mean overall survival times of 299 and 411 days, respectively. The median PFS reported in this study was 220 days considering all the dogs and 780 days and 160 days for group 1 and group 2, respectively, and the mean values were 400, 550, and 150 days, respectively, showing comparable local progression outcomes to the surgery-devoted papers. In addition, ultrasound-guided endoscopic diode laser ablation was tested for TCC of the lower urinary tract with palliative intent in 38 dogs [14]. The median survival time was 380 days for tumors localized only to the urethra, 390 days for tumors in the urethra and trigone, and 473 days for tumors in the body or apex of the bladder, with no involvement of the trigone or urethra.

In conclusion, this study has shown that the adopted protocol is well tolerated, safe, and feasible. The efficacy should be investigated in larger cohort studies for a better comparison with patients undergoing different therapeutic approaches, even if the initial findings regarding both the OST and PFS indicate that the results relative to the local tumor control are at least comparable to those reported in the cited literature. Moreover, in metastatic dogs, a valuable improvement has been observed in both quality of life (with a return to urination in obstructed patients during RT treatment) and in local tumor control.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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