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Management of transitional cell carcinoma of the urinary bladder in dogs: A review

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ABSTRACT

Transitional cell carcinoma (TCC), also referred to as urothelial carcinoma, is the most common form of urinary bladder cancer in dogs, affecting tens of thousands of dogs worldwide each year. Canine TCC is usually a high grade invasive cancer. Problems associated with TCC include urinary tract obstruction, distant metastases in >50% of affected dogs, and clinical signs that are troubling both to the dogs and to their owners. Risk factors for TCC include exposure to older types of flea control products and lawn chemicals, obesity, female sex, and a very strong breed-associated risk. This knowledge is allowing pet owners to take steps to reduce the risk of TCC in their dog.

The diagnosis of TCC is made by histopathology of tissue biopsies obtained by cystoscopy, surgery, or catheter. Percutaneous aspirates and biopsies should be avoided due to the risk of tumor seeding. TCC is most commonly located in the trigone region of the bladder precluding complete surgical resection. Medical treatment is the mainstay for TCC therapy in dogs. Although TCC is not usually curable in dogs, multiple drugs have activity against it. Approximately 75% of dogs respond favorably to TCC treatment and can enjoy several months to a year or more of good quality life. Many promising new therapies for TCC are emerging and with the close similarity between TCC in dogs and high grade invasive bladder cancer in humans, new treatment strategies found to be successful in canine studies are expected to help dogs and to be subsequently translated to humans.

Introduction

Although urinary bladder cancer is reported to comprise only 2% of all reported cancers in the dog (Valli et al., 1995; Knapp, 2006; Knapp and McMillan, 2013; Knapp et al., 2014), bladder cancer affects tens of thousands of dogs every year worldwide. The hospital prevalence or proportionate morbidity of bladder cancer at university-based veterinary teaching hospitals in the United States and Canada has continued to increase over the last 30 years (Knapp et al., 2014). In 2010, dogs with bladder cancer comprised 0.7% of dogs presenting to university teaching hospitals in the Veterinary Medical Database (VMDB) (Knapp et al., 2014). The VMDB is a database of case records in participating veterinary teaching hospitals in the United States and Canada.[1]

Invasive transitional cell carcinoma (TCC), also referred to as invasive urothelial carcinoma, is the most common form of canine urinary bladder cancer (Valli et al., 1995; Knapp, 2006; Knapp and McMillan, 2013; Knapp et al., 2014). Most TCCs are high-grade papillary infiltrative tumors (Valli et al., 1995; Knapp, 2006; Knapp et al., 2014). Other types of bladder cancer occur, but less commonly (Valli et al., 1995; Liptak et al., 2004a; Benigni et al., 2006; Heng et al., 2006; Bae et al., 2007; Kessler et al., 2008; Gelberg, 2010).

Canine TCC is problematic for several reasons and is often locally advanced at the time of diagnosis. Following World Health Organization (WHO) criteria for staging canine bladder tumors (Owen and World Health Organization, 1980) (Table 1), 78% of dogs with TCC have been reported to have T2 tumors (those invading the bladder wall), and 20% to have T3 tumors (those invading neighboring organs) (Knapp and McMillan, 2013; Knapp et al., 2014). The cancer has been reported to involve the prostate in 29% of male dogs (Knapp and McMillan, 2013). The frequent trigonal location and urethral involvement in more than half of dogs can lead to dysuria and partial or complete obstruction of the urinary tract (Knapp and McMillan, 2013; Knapp et al., 2014).

Another major problem in dogs with TCC is distant metastases. As better approaches to control the primary tumor are implemented, and dogs with TCC are living longer, the frequency of distant metastases appears to be increasing (Knapp and McMillan, 2013; Knapp et al., 2014). Distant metastases are typically present in ~20% of cases at diagnosis, and are associated with a worse prognosis (Knapp and McMillan, 2013). More than half of dogs with TCC have distant metastases at death. In a recent report of 137 dogs with TCC undergoing necropsy, distant metastases were documented in 58%

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Table 1
World Health Organization TNM clinical staging system for canine bladder cancer (Owen and World Health Organization, 1980).

T: Primary tumor	
Tis	Carcinoma in situ
T0	No evidence of a primary tumor
T1	Superficial papillary tumor
T2	Tumor invading the bladder wall, with induration
T3	Tumor invading neighboring organs (prostate, uterus, vagina, and pelvic canal)
N: Regional lymph node (internal and external iliac lymph node)	
N0	No regional lymph node involvement
N1	Regional lymph node involved
N2	Regional lymph node and juxtaregional lymph node involved
M: Distant metastases	
M0	No evidence of metastasis
M1	Distant metastasis present

of dogs (Table 2) (Knapp et al., 2014). As discussed below, dogs with TCC are also at high risk for the development of secondary urinary tract infections, and the bacteria involved are becoming increasingly resistant to antibiotic treatment.

TCC in dogs clearly presents many challenges. Fortunately, substantial progress has been made in the management of bladder cancer in dogs, and TCC is considered very 'treatable' (Knapp and McMillan, 2013). Research is setting the stage for further progress against the cancer. Because of the close similarities between dogs and humans with invasive TCC, promising research in dogs is expected to translate into humans to improve the outlook in both species (Knapp et al., 2014). This review summarizes the current opportunities to effectively manage bladder cancer in dogs, including emerging therapies.

Table 2
Sites of metastases in 137 dogs with transitional cell carcinoma (TCC) undergoing necropsy at Purdue University between 2005 and 2013.

Location of metastases	Number of dogs with metastasis at that site (% of 137 dogs undergoing necropsy)
Any metastases	92 (67%)
Any nodal metastases	57 (42%)
Regional nodes (abdominal, pelvic inguinal nodes)	40 (29%)
Thoracic nodes	17 (12%)
Other nodes	1 (1%)
Any distant metastases	80 (58%)
Lung	69 (50%)
Bone	15 (11%)
Liver	10 (7%)
Kidney (metastasis or second primary)	10 (7%)
Adrenal gland	8 (6%)
Skin	8 (6%)
Spleen	6 (4%)
Gastrointestinal tract	3 (2%)
Heart	5 (4%)
Brain	2 (1.5%)

Risk factors and prevention

Multiple factors contribute to the development of TCC in dogs including heritable genetic variants and environmental exposures (Knapp et al., 2014). Risk factors include exposure to older types of flea control products, lawn chemicals and possibly cyclophosphamide, obesity, exposure, and there is a very strong breed-associated and female risk (Glickman et al., 1989, 2004; Raghavan et al., 2004; Bryan et al., 2007; Knapp and McMillan, 2013; Knapp et al., 2014). Information concerning breed-associated risk has recently been updated through analysis of records from the VMDB (Table 3).

The breed-associated risk includes a 21-fold increased risk in Scottish Terriers (STs), and a 3–6.5-fold increased risk in Eskimo dogs, Shetland Sheepdogs, West Highland White Terriers, Keeshonds, Samoyeds, and Beagles compared to mixed breed dogs. Among the less common breeds in the USA, additional breeds appeared to be at increased risk for TCC based on the VMDB analyses, albeit with small numbers of dogs to study. These breeds included Collies, Fox terriers, Airedale terriers, American Eskimo dogs, Chesapeake Bay Retrievers, and Schipperkes.

The female:male ratio of dogs with TCC has been reported to range from 1.71:1 to 1.95:1 (Knapp, 2006; Knapp and McMillan, 2013; Knapp et al., 2014). Interestingly, in dogs with high breed-associated risk, the sex predilection is less pronounced (Knapp et al., 2014). The ratio of female:male dogs was 0.8:1 in STs ($n = 79$), 1.2:1 in Shetland Sheepdogs ($n = 93$), 1.3:1 in West Highland White terriers ($n = 44$), and 1.2:1 in Beagles ($n = 62$) (Knapp et al., 2014). TCC risk is higher in neutered dogs than in intact dogs of both sexes, although the reason for this has not been determined (Bryan et al., 2007; Knapp et al., 2014). Androgen and estrogen receptors have been detected in canine TCC, but their role in the development and progression of bladder cancer is not yet known (Knapp et al., 2014).

Evidence for the link between environmental exposures and TCC risk comes from two case control studies in which owners of dogs with TCC (cases) and of dogs of similar age and breed without TCC (controls) provided information on their dogs' history and potential exposures in order to help identify factors associated with TCC development. In a case control study of dogs of several breeds, an association between TCC and exposure to topical flea and tick dips was noted (Glickman et al., 1989). Newer, spot-on type flea control products appear safer. In a case control study in STs, spot-on products containing fipronil were not associated with increased risk of TCC (Raghavan et al., 2004).

Table 3
Summary of analyses of Veterinary Medical Database records of dogs with transitional cell carcinoma (TCC) and dogs in the same breed without TCC (SNOMED search, 1999–2010) (Knapp et al., 2014). The odds ratios (ORs) of TCC risk compared to the risk in mixed breed dogs are reported for breeds with an OR ≥ 2.0 and at least nine cases of TCC in the breed.

Breed	Number of dogs in that breed in database	TCC cases in that breed in database	OR compared to mixed breed	95% confidence intervals
Mixed breed dog (reference category)	42,777	269	1.0	NA
Scottish terrier	670	79	21.12	16.23–27.49
Eskimo dog	225	9	6.58	3.34–12.96
Shetland Sheepdog	2521	93	6.05	4.76–7.69
West Highland White terrier	1234	44	5.84	4.23–8.08
Keeshond	381	10	4.26	2.25–8.07
Samoyed	471	10	3.43	1.81–6.49
Beagle	3236	62	3.09	2.34–4.08
Dalmatian	1253	19	2.43	1.52–3.89

In a case control study in STs, TCC risk was significantly higher in STs that had been exposed to lawn herbicides alone (odds ratio [OR], 3.62; 95% confidence interval [CI], 1.17–11.19; $P < 0.03$) or herbicides and insecticides (OR, 7.19; 95% CI, 2.15–24.07; $P < 0.001$) than in dogs not exposed to the chemicals (Glickman et al., 2004). Several chemicals were present in the lawn treatment mixture, and the exact chemical(s) responsible for the increased TCC risk was not known. However, the most likely scenario to explain the association between lawn chemical exposure and TCC risk was that chemical carcinogens (or procarcinogens) on the lawn were absorbed by the dogs and excreted in urine, thereby exposing the urothelium to harmful chemicals.

A follow-up study was performed to prospectively measure the concentrations of herbicides in the urine of dogs that lived in households that applied lawn chemicals and of dogs living in control household who did not apply lawn chemicals (Knapp et al., 2013b). Briefly, the concentrations were measured of three chemicals commonly used in commercial lawn care products, namely, 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2methylphenoxypropionic acid (MCPP), and dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl) pyridine-3,5-dicarbothioate (dithiopyr). These chemicals were selected because they would most likely be included in lawn treatment mixtures and could serve as good markers of uptake, not because of specific information about their carcinogenic potential. Concentrations of the three chemicals were measured in the urine of dogs and on the surface of the grass before lawn application, and 24 h and 48 h after chemical application. At least one of the three chemicals was detected in the urine of dogs in 19/25 households after lawn treatment (Knapp et al., 2013b). Of greater concern, however, was that the chemicals were also detected in the urine of dogs in 14/25 households before the lawn was treated and in dogs in 4/8 untreated households. Chemicals were commonly detected in grass residues from treated and untreated lawns, suggesting a chemical drift from nearby treated areas.

The case control study in STs provided a lead on a possible strategy to reduce TCC risk. STs that consumed vegetables at least three times a week, along with their normal diet, had a reduced risk of TCC (OR, 0.30; 95% CI, 0.01–0.97; $P < 0.001$) (Raghavan et al., 2005). The study was not designed to determine the most beneficial vegetable, although carrots, given as treats, were the most frequently fed vegetable in the study.

Pending prospective studies of TCC prevention strategies, a reasonable approach would be to limit exposure to lawn chemicals and older types of flea control products, and to feed vegetables at least three times per week, especially for dogs in breeds with high risk for TCC. The owners of dogs in high-risk breeds should be made aware of the TCC risk, instructed to take note of suspicious urinary tract signs, and to pursue timely veterinary care if signs are noted. Prospective studies to determine the value of TCC screening and early detection are in progress.

Clinical presentation and diagnosis

The most common clinical signs in dogs with TCC are hematuria, stranguria, and pollakiuria, and these signs can be present for weeks to months prior to diagnosis (Knapp and McMillan, 2013). Antibiotic treatment may cause reduction or temporary resolution of clinical signs. Apparent tenesmus may result from physical or neurological abnormalities. Less commonly, dogs can have lameness caused by bone metastasis or hypertrophic osteopathy (Knapp and McMillan, 2013). The urinary tract signs with TCC closely mimic those of a urinary tract infection. Since the vast majority of dogs with these signs have urinary tract infections, in most dogs it is prudent to find and treat the infection first if it is present. Should infection not be present, or should clinical signs persist or recur after treatment for an infection, then other possible urinary tract abnormalities such as cancer and stones should be investigated. In middle age to older dogs in breeds with high risk for TCC, however, it is appropriate to evaluate these dogs for TCC on initial presentation with urinary tract signs.

Finding abnormal epithelial cells in urine and thickened bladder wall or mass lesions within the urinary tract also increases suspicion for TCC; however, these findings can occur with other conditions too. A urine antigen test for TCC has been found to be sensitive (Henry et al., 2003b), but false-positive results have limited the value of this test in dogs presenting with locally advanced cancer. Differential diagnoses include other neoplasia, chronic cystitis, polypoid cystitis, fibroepithelial polyps, granulomatous cystitis and urethritis, gossypiboma, calculi, and inflammatory pseudotumor (Owen and World Health Organization, 1980; Valli et al., 1995; Martinez et al., 2003; Liptak et al., 2004a, 2004b; Benigni et al., 2006; Heng et al., 2006; Bae et al., 2007; Kessler et al., 2008; Deschamps and Roux, 2009; Böhme et al., 2010; Gelberg, 2010). It is important to distinguish non-TCC conditions from TCC because the treatment and prognosis differ considerably and depend on the diagnosis.

Histopathological examination provides a definitive diagnosis of TCC and characterization of the different pathological types of TCC (Valli et al., 1995; Patrick et al., 2006; Knapp et al., 2014). Tissue biopsies from the bladder can be obtained by cystotomy, cystoscopy, or traumatic catheterization (Messer et al., 2005; Holak et al., 2007; Childress et al., 2011; Knapp et al., 2014). With cystoscopy, the operator can visually inspect the urethra and bladder and obtain biopsies using a noninvasive method. With the small size of cystoscopic biopsies, however, diligence is required to obtain sufficient tissue for diagnosis. The use of a histology cassette can help prevent loss of small samples during processing. In 92 dogs, diagnostic samples were obtained by cystoscopy in 96% of female dogs and 65% of male dogs that ultimately had TCC diagnosed by histopathology (Childress et al., 2011). The use of a wire stone collection basket during cystoscopy allows for collection of larger tissue samples, and has increased the yield of diagnostic biopsies. Traumatic catheterization to collect tissues for diagnosis can also be performed, although the quality of samples obtained varies. TCC has a notable capacity to grow in the abdominal wall if seeded there, and percutaneous biopsy methods should, therefore, be avoided (Nyland et al., 2002; Vignoli et al., 2007).

Immunohistochemistry for uroplakin III (UPIII) can be used to help distinguish TCC from other poorly differentiated carcinomas (Ramos-Vara et al., 2003; Sledge et al., 2014) . UPIII is expressed in >90% of canine TCC (Ramos-Vara et al., 2003). Although it has been considered a specific marker for TCC, there are reports of UPIII expression in canine prostate cancer (Ramos-Vara et al., 2003; Lai et al., 2008).

Clinical evaluation and staging

The physical examination of dogs with TCC should include a rectal examination. Physical examination findings can include thickening of the urethra and trigone region of the bladder, iliac and pelvic lymphadenopathy, and sometimes a mass in the bladder or a distended bladder (Knapp and McMillan, 2013). Although uncommon, TCC can metastasize to the skin resulting in erythematous, ulcerated, or proliferative lesions (Reed et al., 2013). A normal physical examination does not rule out TCC.

In dogs with confirmed or suspected TCC, evaluation should include an assessment of overall health and tests to determine tumor stage, as this information will be used in planning treatment (Knapp and McMillan, 2013). This includes a complete blood count (CBC), serum biochemistry profile, urinalysis with or without urine culture, thoracic radiography, abdominal ultrasonography, and urinary tract imaging. Urine should be collected by free catch or catheterization;

cystocentesis should be avoided as it could lead to tumor seeding. Catheter placement must be performed carefully to avoid perforation of the diseased bladder or urethral wall. Thoracic radiography and abdominal ultrasonography can be performed to detect metastases in lymph nodes, liver, lungs, and other sites (Table 2) (Knapp, 2006; Gelberg, 2010; Colledge et al., 2013; Knapp and McMillan, 2013; Pancotto et al., 2013; Reed et al., 2013). If unexplained lameness is present, radiography or scintigraphy to detect possible bone metastasis should be considered.

Urinary tract imaging is used to assess the primary tumor location for potential surgical intervention, to provide prognostic information, and to map and measure TCC masses in order to subsequently monitor response to medical therapy (Chun et al., 1997; Hume et al., 2010; Naughton et al., 2012; Hanazono et al., 2013). Methods to map the TCC in the bladder, proximal urethra, and prostate include cystosonography, cystography, or computed tomography (CT) (Chun et al., 1997; Naughton et al., 2012). The preferred method for monitoring tumor response at our institution is ultrasonography using a standardized protocol which can accurately be performed in awake dogs in a timely and cost-effective fashion.

When using ultrasonography to monitor changes in TCC masses, however, it is essential (1) to have the same operator perform each examination; (2) to standardize the dog's position, probe position, and data collection, and (3) to have a similar level of bladder distension for each ultrasound examination visit. When compared to two-dimensional ultrasound measurements, three-dimensional ultrasound measurements of bladder lesions have been found to more closely mimic those from CT, and to be less affected by the degree of bladder distension (Naughton et al., 2012).

Regardless of the imaging technique used, it is crucial to have a standardized protocol to accurately track tumor response. It is important to use imaging to monitor tumor response; clinical signs alone are not sufficient because signs can change dramatically with the presence or resolution of urinary tract infection.

Treatment

Surgery and interventional urology procedures

Surgery may be indicated in dogs with TCC (1) to obtain tissue for a diagnosis; (2) to attempt to remove the TCC within the bladder when the cancer is away from the trigone, and (3) to restore or maintain urine flow (Knapp and McMillan, 2013). The potential value of cytoreductive surgery to enhance the activity of adjuvant therapy has not yet been defined. If surgery is performed, it is crucial to take measures to avoid seeding the cancer into surrounding structures and the abdominal wall.

In a recent report, 24/544 dogs with TCC evaluated at the Purdue University Veterinary Teaching Hospital (PUVTH) had spread of TCC to the abdominal wall (Higuchi et al., 2013). TCC in the abdominal wall developed significantly more often in dogs that had undergone cystotomy (18/177, 10.2%) than in those that had not (6/367, 1.6%). Once detected in the abdominal wall, the TCC grew aggressively and did not respond to medical therapy. The median survival after detection of the abdominal wall TCC in the 24 dogs was 57 days (range, 0–324 days) (Higuchi et al., 2013). This report also demonstrated that (although uncommon) TCC can spread to the abdominal wall via lymphatics. With the poor outlook for dogs with abdominal wall TCC, every effort should be made to avoid seeding the tumor. Therefore, careful techniques should be applied when performing surgery.

It is usually not possible to completely excise TCC from the bladder because of the typical trigonal location, urethral involvement, and in some cases, metastases. Techniques for trigone resection (Saulnier-Troff et al., 2008) or cystectomy (Stratmann and Wehrend, 2007; Hautmann, 2008) and the use of grafting materials to replace bladder tissues (Zhang et al., 2006; Wongsetthachai et al., 2011) have been reported in the dog, but these approaches have not been used to any extent because of morbidity and expense. An additional factor when considering surgical resection of TCC is that many dogs develop multifocal TCC in the bladder like in humans (Harris and Neal, 1992; Azémar et al., 2011). This synchronous or metachronous development of multiple TCC masses could be due to a 'field' effect in which the entire bladder lining is thought to undergo neoplastic change in response to carcinogens, or through intraluminal seeding and implantation of tumor cells (Azémar et al., 2011). In a series of 67 dogs with TCC that underwent surgery for biopsy or for therapeutic intent, complete surgical excision of the tumor with tumor-free margins was possible in only two dogs (Knapp and McMillan, 2013). One of the two dogs had a relapse in the bladder 8 months later, and the second dog developed metastatic disease.

Although curative-intent surgery has a limited role in dogs with TCC, surgery can have an important role in restoring or maintaining urine flow. Ureteral stents, when needed, have traditionally been placed surgically, although less invasive approaches for stent placement have been reported (Berent, 2011). Ureterocolonic anastomosis has been described, but is not recommended due to the high complication rate and limited survival (Stone et al., 1988). Prepubic cystostomy catheters can be an effective means to bypass urethral obstruction (Smith et al., 1995; Salinardi et al., 2003). In recent years, the placement of urethral stents has been pursued rather than cystostomy tubes because there are not any external components of stents for the dog to chew out, and the pet owner does not have to drain the bladder as with cystostomy tubes (Weisse et al., 2006; McMillan et al., 2012; Blackburn et al., 2013). Urethral stents can be placed non-surgically with fluoroscopic guidance (McMillan et al., 2012; Blackburn et al., 2013).

The outcome for dogs with TCC and urethral stents has recently been summarized (McMillan et al., 2012; Blackburn et al., 2013). Stent placement was successful in relieving urethral obstruction in 58/61 dogs in two studies. In one dog, the tumor was so extensive that the stent could not be advanced up the urethra. In a second dog, the stent collapsed immediately after placement due to tumor compression. In a third dog, the stent did not stay in place due to the suboptimal size of the stent. Stent placement can be lifesaving in the majority of dogs with urethral obstruction, but complications can occur. Incontinence was reported in 39% and 26% of dogs in these studies (McMillan et al., 2012; Blackburn et al., 2013). Other complications included stent migration and re-obstruction due to further tumor growth (McMillan et al., 2012). Urethral stents could also theoretically increase the risk of urinary tract infection. The median survival after stent placement was 78 days (range, 2–537 days) (McMillan et al., 2012; Blackburn et al., 2013). A survey of the owners of dogs with stents indicated that 16/17 owners were satisfied with the outcome and would recommend stent placement to other owners (McMillan et al., 2012).

Another interventional approach reported to address urethral tumors is transurethral resection (Liptak et al., 2004b; Cerf and Lindquist, 2012). Pet owners may inquire about transurethral resection because of awareness of the common application of this technique in human bladder cancer. Briefly, in a cystoscopic procedure, instruments are passed to remove tumor tissue from the bladder and urethra. What most pet owners do not recognize, however, is that this approach is most often applied to superficial tumors in humans where it is possible to resect the lesions cystoscopically without penetrating the bladder or urethral wall. Unfortunately, TCC in most dogs is invasive into the bladder wall. A procedure has been reported that involves the use of ultrasound to attempt to guide endoscopic diode laser ablation of TCC (Cerf and Lindquist, 2012). Using this and similar approaches, the main risk of laser resection of TCC in dogs remains bladder perforation. The benefit of laser resection has not been well defined as dogs in

previous reports were receiving multi-modality treatment, and it is not possible to know which component(s) of the treatment could have been beneficial.

Radiation therapy

Initial reports of the use of radiation therapy (RT) in TCC and other bladder tumors were limited (Walker and Breider, 1987; Withrow et al., 1989). Complications were common and included cystitis, urinary incontinence, colitis, and in some cases colonic stricture. Other studies have confirmed complications associated with pelvic irradiation (Anderson et al., 2002). In a more recent report of 10 dogs, weekly coarse fraction external-beam RT combined with mitoxantrone and piroxicam was tolerated, but results were not better than those with medical therapy alone (Poirier et al., 2004). It is challenging to deliver RT to the bladder because the bladder is not rigidly fixed in place in the abdomen and can flop from side to side. As the bladder fills and empties, it changes size, location, and shape. As material moves through the intestinal tract, the intestine can press on the bladder causing change in bladder location or shape. Therefore, in earlier studies, the treatment field was relatively large. Expanding expertise and equipment, however, are making it much more feasible to direct RT to the bladder, urethra, and prostate, while sparing normal tissues, especially when onboard imaging is available for use with each radiation dose (Nolan et al., 2012; Nieset et al., 2014). This can set the stage for follow-up studies to determine the benefits of state-of-the-art RT.

Medical therapy

The mainstay of TCC treatment in dogs continues to be systemic medical therapy which usually consists of chemotherapy, cyclooxygenase (COX) inhibitors (non-selective COX inhibitors and COX-2 inhibitors), and combinations of these (Table 4) (Moore et al., 1990; Chun et al., 1996; Knapp et al., 2000, 2013a, 2014; Henry et al., 2003b; Mohammed et al., 2003; Boria et al., 2005; Greene et al., 2007; Arnold et al., 2011; Marconato et al., 2011; McMillan et al., 2011; Hahn et al., 2012; Dhawan et al., 2013a; Knapp and McMillan, 2013; Robat et al., 2013; Schrempp et al., 2013). Although medical therapy is not usually curative, remission or stable disease (lack of progression) can be accomplished with several different drugs, and most treatments are well tolerated (Knapp and McMillan, 2013). If resistance to one drug develops, other drugs can still be effective. The best results often occur in dogs that sequentially receive multiple different treatment protocols over the course of their disease.

At our hospital, the approach used is to obtain baseline measurements of the TCC masses, to initiate a starting treatment, to monitor the response to that treatment at 4- to 8-week intervals, and to continue that treatment as long as the TCC is controlled, side effects are absent or acceptable, and quality of life is good. Although remission is obviously the preferred response, in many dogs a treatment response of stable disease (either no change in tumor size or a change that is less than what would be classified as remission or progressive disease) may be considered a positive outcome if clinical signs are acceptable and the dog is enjoying quality life. A different treatment is instituted if cancer progression or unacceptable toxicity occurs. Further changes in treatment are based on tumor response and treatment tolerability.

When using this approach, TCC growth can be controlled in approximately 75% of dogs, quality of life is usually very good, and median survival times can extend well beyond a year. Although the question could be raised as to whether it would be more appropriate to simultaneously combine multiple chemotherapy agents in dogs with TCC, the benefit of this has not been determined. In addition, giving multiple chemotherapeutic agents together is still unlikely to be curative, would be expected to be more toxic, and could lead to the development of resistance to multiple drugs at the same time limiting options for subsequent therapy.

Table 4
Results of medical therapy of transitional cell carcinoma (TCC) in dogs.

Drug and reference	Number of dogs	% Remission (complete and partial)[a]	% Stable disease[a]	Median survival (days)[b]
Piroxicam (Knapp et al., 2014)	76	21	59	244
Deracoxib (McMillan et al., 2011)	24	17	71	323
Mitoxantrone/piroxicam (Henry et al., 2003b)	48	35	46	291
Vinblastine (Arnold et al., 2011)	28	36	50	147
Vinblastine–folate conjugate (Dhawan et al., 2013a)	9	56	44	115
Cisplatin (50–60 mg/m^2) (Moore et al., 1990; Chun et al., 1996, 1997; Knapp et al., 2000, 2013b; Boria et al., 2005; Greene et al., 2007)	28	19–25	25–50	105–130
Cisplatin (60 mg/m^2)/piroxicam (Mohammed et al., 2003)	12	50	17	329
Carboplatin (Chun et al., 1997)	12	0	8	132
Carboplatin/piroxicam (Boria et al., 2005)	29	38	45	161
Gemcitabine/piroxicam (Marconato et al., 2011)	38	26	50	230
Doxorubicin/piroxicam (Robat et al., 2013)	23	9	60	168
5-aza-citadine (Hahn et al., 2012)	18	22	50	203
Leukeran, 'metronomic' (4 mg/m^2) (Schrempp et al., 2013)	30	3	67	221
Randomized trial[c] (Knapp et al., 2000)				
Cisplatin (60 mg/m^2)	8	0	50	300
Cisplatin (60 mg/m^2)/piroxicam	14	71	28	246
Randomized trial[d] (Knapp et al., 2013a)				
Cisplatin (60 mg/m^2)	15	13	53	338
Firocoxib	15	20	33	152
Cisplatin (60 mg/m^2)/firocoxib	14	57	21	179

[a] Complete remission refers to the resolution of all clinical evidence of TCC. Partial remission is typically defined as ≥50% reduction in tumor volume, but is defined for each study in the referenced publication. Stable disease is a response less than remission or progressive disease, and is more specifically defined in each study publication.

[b] Survival is measured from the time the study drug was initiated until death. Survival from diagnosis to death could be longer than the indicated time if other therapies had been given between diagnosis and the initiation of study drug. Similarly, drugs given after the study drug period could influence survival.

[c] Dogs were randomized to receive either cisplatin alone or cisplatin combined with piroxicam. If cancer progression occurred in dogs receiving cisplatin alone, the dogs then received piroxicam alone. The survival time of 300 days for the cisplatin group reflects survival following cisplatin and then piroxicam.

[d] Dogs were randomized to receive cisplatin alone, firocoxib alone, or the two drugs combined. When dogs receiving single agent therapy experienced cancer progression or unacceptable toxicity, the dogs were then eligible to receive the other single agent drug. Of 15 dogs treated with cisplatin alone, 13 went on to receive firocoxib alone, and the median survival from start of the first drug was 338 days. Of 15 dogs receiving firocoxib alone, five dogs went on to receive cisplatin alone, and the median survival was 152 days.

As summarized in Table 4, several different drugs have activity against TCC in dogs, and there are many acceptable treatment options. The most commonly used intravenous (IV) drugs have been mitoxantrone and vinblastine, with gemcitabine also showing recent promise (Henry et al., 2003a; Arnold et al., 2011; Marconato et al., 2011; Knapp and McMillan, 2013). These drugs result in remission in ~35% of dogs and in stable disease in ~45–50% of dogs, and are generally well tolerated. The platinum compounds (cisplatin, carboplatin) appear to be the most active agents, but are more likely to cause side effects (Moore et al., 1990; Chun et al., 1996, 1997; Knapp et al., 2000, 2013a; Boria et al., 2005; Greene et al., 2007).

Cisplatin appears to have the greatest level of antitumor activity against canine TCC, especially when combined with a COX inhibitor (Knapp et al., 2000, 2013a; Greene et al., 2007). The combination of cisplatin and the non-selective COX inhibitor, piroxicam, led to impressive remission rates (50–71%), but renal, gastrointestinal, and bone marrow toxicities limit the use of this protocol (Knapp et al., 2000; Greene et al., 2007). Renal toxicity has been especially problematic when cisplatin is combined with piroxicam (Knapp et al., 2000; Greene et al., 2007). Firocoxib (Previcox, Merial), a selective COX-2 inhibitor, has been successfully combined with cisplatin without increasing renal toxicity, although the inherent toxicity of cisplatin still limits this approach (Knapp et al., 2013a). Interestingly, in two randomized trials, the most impressive median survival times (300 and 338 days, respectively) were for the dogs who initially received cisplatin alone, and then when that treatment failed (due to toxicity or tumor progression) they received a COX inhibitor alone (Knapp et al., 2000, 2013a), although toxicity still limits the use of cisplatin. Additional work to confirm these results and to understand mechanisms involved is warranted.

If dog owners wish to pursue a more conservative treatment approach and limit therapy to oral agents, then COX inhibitors offer a good option. As a single agent, oral piroxicam (0.3 mg/kg daily with food) is a useful palliative treatment for dogs with TCC providing excellent quality of life (Knapp and McMillan, 2013; Knapp et al., 2014). Tumor responses in 76 dogs with TCC that received piroxicam as a single agent included two (2.6%) complete remission (complete resolution of all clinical evidence of cancer), 14 (18.4%) partial remission (≥50% reduction in tumor volume and no new tumor lesions), 45 (59.2%) stable disease (<50% change in tumor volume and no new lesions), and 15 (19.7%) progressive disease (≥50% increase in tumor volume of the development of new tumor lesions) (Knapp et al., 2014). The median survival time (244 days) compared favorably to that of 55 dogs in the Purdue Comparative Oncology Program Tumor Registry that were treated with cytoreductive surgery alone (109 days) (Knapp and McMillan, 2013).

Although most dogs tolerate piroxicam well, care must be taken to detect gastrointestinal toxicity, particularly ulceration. If vomiting, melena, and anorexia occur, the drug must be withdrawn and supportive care provided as needed until the toxicity resolves. In these cases, it may be safest to switch to a selective COX-2 inhibitor if further COX inhibitor treatment is indicated.

There is evidence that selective COX-2 inhibitors also have antitumor activity against TCC in dogs. The selective COX-2 inhibitor, deracoxib (Deramaxx, Novartis Animal Health), given as a single agent (3 mg/kg/day orally) resulted in remission in 17% of dogs and stable disease in 71% of dogs with TCC (McMillan et al., 2011). In a randomized trial, firocoxib induced remission in 20% of dogs as a single agent, and enhanced the activity of cisplatin from 13% (single agent cisplatin) to 57% (combination) (Knapp et al., 2013a). The remission rate with selective COX-2 inhibitors appears similar to that observed with piroxicam; however, occasional complete remissions occur in dogs receiving piroxicam, whereas this has not been reported in dogs treated with deracoxib or firocoxib. Deracoxib has also shown promise in a pilot study of adjuvant COX inhibitor treatment following surgical resection of TCC in nine dogs (Knapp and McMillan, 2013). Of three dogs with tumor-free margins, recurrence (consistent with the field effect) was noted in two dogs at 210 and 332 days, respectively; the third dog died at 1437 days with no evidence of relapse. Two of six dogs with microscopic residual TCC after surgery relapsed at 140 and 231 days, respectively. No relapse was detected in the other four dogs at the time of death (345, 749, and 963 days), or at 2057 days in a dog which was still alive. The median survival of the nine dogs was 749 days (range, 231–2581 days).

Intravesical therapy

In addition to systemically delivered therapy, intravesical therapy has been investigated in dogs with bladder confined TCC. Intravesical therapy is commonly used in humans with superficial TCC, and there has been some interest in testing this approach to potentially treat the higher grade invasive TCC in dogs if it has not spread beyond the bladder (Abbo et al., 2010). A phase I clinical trial and pharmacokinetic (PK) study of intravesical mitomycin C (MMC, 1 h dwell time/day, two consecutive days each month, escalating concentrations) was performed in dogs with TCC (Abbo et al., 2010). Tumor responses included partial remission in 5/13 dogs, and stable disease in 7/13 dogs. Although the drug was initially well tolerated, severe myelosuppression and gastrointestinal upset subsequently occurred in two dogs, most likely due to systemic absorption of the drug from the bladder. Both dogs recovered with supportive care, but high systemic exposure is of great concern: the dose administered in the bladder is sufficiently high that, if a substantial proportion were to be systemically absorbed, it could be lethal. If other drugs have failed and no other options are available, the clinician and owner need to carefully consider the risk vs. potential benefit of attempting intravesical therapy. Other intravesical therapies may emerge for use in dogs with TCC (Lu et al., 2011).

Emerging treatment strategies

In addition to traditional cytotoxic chemotherapy agents and COX inhibitors, other treatment strategies are emerging or are under investigation including metronomic chemotherapy, targeted cytotoxic therapy, and demethylating agent therapy. Metronomic chemotherapy is the administration of low doses of chemotherapeutic drugs frequently with the goal to delay or prevent cancer progression. It is expected to be less toxic, to lead to stable disease, and to work through multiple mechanisms (Schrempp et al., 2013). In a study of metronomic oral chlorambucil (4 mg/m^2 daily) in 31 dogs with TCC, one dog (3%) had partial remission and 20 dogs (67%) had stable disease. Of 31 dogs enrolled in the study, 29 dogs had failed previous therapies. The median progression free interval and survival from the start of chlorambucil treatment were 119 days (range, 7–728 days) and 221 days (range, 7–747 days), respectively. Quality of life was excellent, and toxicity was minimal, although myelosuppression has occasionally been observed in dogs treated with the same dose after the study, and hematological monitoring is recommended.

Targeted cancer therapy is receiving tremendous interest because it is expected to offer the greatest antitumor effects with the least risk of toxicity, and to allow for more individualized cancer treatment. One example of a targeted therapy approach that has been investigated in dogs with TCC is 'folate-targeted therapy', or more specifically treatment targeted to folate receptors. This approach exploits the high uptake of folate (vitamin B9) and folate drug conjugates into certain cancers that express high affinity folate receptors (Dhawan et al., 2013a).

Immunohistochemistry and scintigraphy suggest that approximately 75% of dogs with TCC will have high folate uptake in their

cancer. In a dose escalation study of folate-targeted vinblastine (EC0905, Endocyte), the tumor responses in nine dogs included partial remission in five dogs and stable disease in four dogs (Dhawan et al., 2013a). The drug was generally well tolerated with myelosuppression and gastrointestinal upset being the dose limiting toxicities at higher doses. Other targeting strategies are also being investigated in canine TCC such as utilizing a bladder cancer specific peptide, PLZ4, which recognizes human and canine TCCs (Lin et al., 2011; Zhang et al., 2012).

New strategies for cancer therapy are also being developed around epigenetic events that can drive cancer development and progression in the absence of DNA mutations (Baylin and Jones, 2011). A major event in this process is aberrant methylation in the promoter region of tumor suppressor genes, resulting in gene silencing. Aberrant DNA methylation in multiple cancer-related genes has been found in human TCC tissues and cell lines (Besaratinia et al., 2013). A key enzyme in DNA methylation (DNMT1) has been found to be overexpressed in human and canine TCC (Dhawan et al., 2013b). In a clinical trial of the demethylating agent, 5-azacitidine (given by daily subcutaneous (SC) injection for five consecutive days once or twice per month) in dogs with TCC, partial remission, stable disease, and progressive disease were observed in four (22.2%), nine (50.0%), and four (22.2%) dogs, respectively, demonstrating the promising activity of a demethylating agent in dogs with TCC (Hahn et al., 2012). Follow-up work is in progress with an oral demethylating agent (zebularine) in dogs, and preliminary findings have been presented (Fulkerson et al., 2013). It is expected that demethylating agents will need to be given daily, and the oral formulation of zebularine provides an advantage over other agents that are only available in injectable forms. Zebularine results in potent myelosuppression at high doses in dogs, but lower doses are well tolerated. Control of TCC has been achieved in the majority of dogs studied to date (Fulkerson et al., 2013).

Secondary urinary tract infections and increasing challenges

Dogs with TCC are at high risk of secondary bacterial infections as the cancer leads to abnormal urination and urine retention, acquired anatomical 'defects' in the bladder and urethra, altered urothelium, and in some cases potentially compromised immune function (Thompson et al., 2011).

Urinary tract infections in dogs with TCC are problematic for several reasons. Infection leads to a marked worsening of urinary tract signs, impacting quality of life, leading to concerns for owners, and potentially giving the false impression that the cancer is progressing. Another major problem is that bacteria are becoming increasingly resistant to antibiotics (Thompson et al., 2011; Boothe et al., 2012; Hall et al., 2013; Nam et al., 2013; Wagner et al., 2014). Of 57 dogs recently treated for TCC over a 12-month period at the PUVTH, seven dogs developed highly resistant infections that were only sensitive to nephrotoxic or very expensive antibiotics, or in some cases not sensitive to any antibiotics evaluated. This can lead to euthanasia even when the cancer is controlled if the antibiotic therapy is ineffective, poorly tolerated, or too costly.

There is also concern that persistent or recurrent urinary tract infections can enhance tumor progression and diminish treatment response. Bacterial infection leads to marked inflammation, which can play a crucial role at several different stages of tumor development, including initiation, promotion, malignant transformation, invasion, and metastasis (Grivennikov et al., 2010; Chung et al., 2013; Iida et al., 2013; Ohnishi et al., 2013). Tumor-associated inflammatory cells and cytokines contribute to malignant transformation and promote cancer cell proliferation (Grivennikov et al., 2010; Iida et al., 2013).

There is also recent evidence that antibiotic use can contribute to cancer progression. In mice bearing implanted tumor xenografts, antibiotic treatment resulted in gene changes in cancer cells which were associated with decreased antigen presentation, decreased phagocytosis by immune cells, decreased response to immunotherapy and chemotherapy, and increased expression of genes associated with cell proliferation (Iida et al., 2013). The diminished response to immunotherapy was thought to be due to changes in commensal microbiota (gastrointestinal flora) which would normally be important in priming tumor-associated innate myeloid cells to respond to the immunotherapy (Iida et al., 2013).

For these reasons, periodic urinalyses are indicated in dogs with TCC, and urine culture and sensitivity tests should be performed when infection is suspected. Antibiotics should be selected based on these results, and the urine re-cultured after conclusion of the course of antibiotics. It has not yet been established if periodic urine culture in the absence of worsening clinical signs or pyuria is useful or not. This approach could allow for detection of early pathogenic infection, but could also lead to the detection of nonpathogenic bacteria that may not require treatment.

Conclusions

Much progress has been made in the management of TCC, and this cancer is a highly treatable condition with expected good outcome in the majority of dogs. TCC, however, is still not curable in most cases. Research is continuing to define more effective strategies for the prevention, early detection, and treatment of TCC in dogs, with potential to translate successful findings into human studies.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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References

Abbo, A.H., Jones, D.R., Masters, A.R., Stewart, J.C., Fourez, L., Knapp, D.W., 2010. Phase I clinical trial and pharmacokinetics of intravesical mitomycin C in dogs with localized transitional cell carcinoma of the urinary bladder. Journal of Veterinary Internal Medicine 24, 1124–1130.

Anderson, C.R., McNiel, E.A., Gillette, E.L., Powers, B.E., LaRue, S.M., 2002. Late complications of pelvic irradiation in 16 dogs. Veterinary Radiology and Ultrasound 43, 187–192.

Arnold, E.J., Childress, M.O., Fourez, L.M., Tan, K.M., Stewart, J.C., Bonney, P.L., Knapp, D.W., 2011. Clinical trial of vinblastine in dogs with transitional cell carcinoma of the urinary bladder. Journal of Veterinary Internal Medicine 25, 1385–1390.

Azémar, M.-D., Comperat, E., Richard, F., Cussenot, O., Rouprêt, M., 2011. Bladder recurrence after surgery for upper urinary tract urothelial cell carcinoma: Frequency, risk factors, and surveillance. Urologic Oncology 29, 130–136.

Bae, I.-H., Kim, Y., Pakhrin, B., You, M.-H., Hwang, C.-Y., Kim, J.-H., Kim, D.-Y., 2007. Genitourinary rhabdomyosarcoma with systemic metastasis in a young dog. Veterinary Pathology 44, 518–520.

Baylin, S.B., Jones, P.A., 2011. A decade of exploring the cancer epigenome – Biological and translational implications. Nature Reviews. Cancer 11, 726–734.

Benigni, L., Lamb, C.R., Corzo-Menendez, N., Holloway, A., Eastwood, J.M., 2006. Lymphoma affecting the urinary bladder in three dogs and a cat. Veterinary Radiology and Ultrasound 47, 592–596.
Berent, A.C., 2011. Ureteral obstructions in dogs and cats: A review of traditional and new interventional diagnostic and therapeutic options. Journal of Veterinary Emergency and Critical Care 21, 86–103.
Besaratinia, A., Cockburn, M., Tommasi, S., 2013. Alterations of DNA methylome in human bladder cancer. Epigenetics 8, 1013–1022.
Blackburn, A.L., Berent, A.C., Weisse, C.W., Brown, D.C., 2013. Evaluation of outcome following urethral stent placement for the treatment of obstructive carcinoma of the urethra in dogs: 42 cases (2004–2008). Journal of the American Veterinary Medical Association 242, 59–68.
Boothe, D., Smaha, T., Carpenter, D.M., Shaheen, B., Hatchcock, T., 2012. Antimicrobial resistance and pharmacodynamics of canine and feline pathogenic E. coli in the United States. Journal of the American Animal Hospital Association 48, 379–389.
Boria, P.A., Glickman, N.W., Schmidt, B.R., Widmer, W.R., Mutsaers, A.J., Adams, L.G., Snyder, P.W., DiBernardi, L., de Gortari, A.E., Bonney, P.L., et al., 2005. Carboplatin and piroxicam therapy in 31 dogs with transitional cell carcinoma of the urinary bladder. Veterinary and Comparative Oncology 3, 73–80.
Böhme, B., Ngendahayo, P., Hamaide, A., Heimann, M., 2010. Inflammatory pseudotumours of the urinary bladder in dogs resembling human myofibroblastic tumours: A report of eight cases and comparative pathology. The Veterinary Journal 183, 89–94.
Bryan, J.N., Keeler, M.R., Henry, C.J., Bryan, M.E., Hahn, A.W., Caldwell, C.W., 2007. A population study of neutering status as a risk factor for canine prostate cancer. The Prostate 67, 1174–1181.
Cerf, D.J., Lindquist, E.C., 2012. Palliative ultrasound-guided endoscopic diode laser ablation of transitional cell carcinomas of the lower urinary tract in dogs. Journal of the American Veterinary Medical Association 240, 51–60.
Childress, M.O., Adams, L.G., Ramos-Vara, J.A., Freeman, L.J., He, S., Constable, P.D., Knapp, D.W., 2011. Results of biopsy via transurethral cystoscopy and cystotomy for diagnosis of transitional cell carcinoma of the urinary bladder and urethra in dogs: 92 cases (2003–2008). Journal of the American Veterinary Medical Association 239, 350–356.
Chun, R., Knapp, D.W., Widmer, W.R., Glickman, N.W., DeNicola, D.B., Bonney, P.L., 1996. Cisplatin treatment of transitional cell carcinoma of the urinary bladder in dogs: 18 cases (1983–1993). Journal of the American Veterinary Medical Association 209, 1588–1591.
Chun, R., Knapp, D.W., Widmer, W.R., DeNicola, D.B., Glickman, N.W., Kuczek, T., Degortari, A., Han, C.M., 1997. Phase II clinical trial of carboplatin in canine transitional cell carcinoma of the urinary bladder. Journal of Veterinary Internal Medicine 11, 279–283.
Chung, S.-D., Tsai, M.-C., Lin, C.-C., Lin, H.-C., 2013. A case-control study on the association between bladder cancer and prior bladder calculus. BMC Cancer 13, 117.
Colledge, S.L., Raskin, R.E., Messick, J.B., Tiffany Reed, L., Wigle, W.L., Balog, K.A., 2013. Multiple joint metastasis of a transitional cell carcinoma in a dog. Veterinary Clinical Pathology 42, 216–220.
Deschamps, J.-Y., Roux, F.A., 2009. Extravesical textiloma (gossypiboma) mimicking a bladder tumor in a dog. Journal of the American Animal Hospital Association 45, 89–92.
Dhawan, D., Ramos-Vara, J.A., Naughton, J.F., Cheng, L., Low, P.S., Rothenbuhler, R., Knapp, D.W., 2013a. Targeting folate receptors to treat invasive urinary bladder cancer. Cancer Research 73, 875–884.
Dhawan, D., Ramos-Vara, J.A., Hahn, N.M., Waddell, J., Olbricht, G.R., Zheng, R., Knapp, D.W., 2013b. DNMT1: An emerging target in the treatment of invasive urinary bladder cancer. Urologic Oncology 31, 1761–1769.
Fulkerson, C., Dhawan, D., Jones, D., Fourez, L., Bonney, P., Knapp, D.W., 2013. Pharmacokinetics and toxicity of the novel oral demethylating agent zebularine in laboratory and tumor-bearing dogs. In: Proceedings of the Annual Meeting of the Veterinary Cancer Society, pp. 41.
Gelberg, H.B., 2010. Urinary bladder mass in a dog. Veterinary Pathology 47, 181–184.
Glickman, L.T., Schofer, F.S., McKee, L.J., Reif, J.S., Goldschmidt, M.H., 1989. Epidemiologic study of insecticide exposures, obesity, and risk of bladder cancer in household dogs. Journal of Toxicology and Environmental Health 28, 407–414.
Glickman, L.T., Raghavan, M., Knapp, D.W., Bonney, P.L., Dawson, M.H., 2004. Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. Journal of the American Veterinary Medical Association 224, 1290–1297.
Greene, S.N., Lucroy, M.D., Greenberg, C.B., Bonney, P.L., Knapp, D.W., 2007. Evaluation of cisplatin administered with piroxicam in dogs with transitional cell carcinoma of the urinary bladder. Journal of the American Veterinary Medical Association 231, 1056–1060.
Grivennikov, S.I., Greten, F.R., Karin, M., 2010. Immunity, inflammation, and cancer. Cell 140, 883–899.
Hahn, N.M., Bonney, P.L., Dhawan, D., Jones, D.R., Balch, C., Guo, Z., Knapp, D.W., 2012. Subcutaneous 5-azacitidine treatment of naturally occurring canine urothelial carcinoma: A novel epigenetic approach to human urothelial carcinoma drug development. The Journal of Urology 187, 302–309.
Hall, J.L., Holmes, M.A., Baines, S.J., 2013. Prevalence and antimicrobial resistance of canine urinary tract pathogens. The Veterinary Record 173, 549.
Hanazono, K., Fukumoto, S., Endo, Y., Ueno, H., Kadosawa, T., Uchide, T., 2013. Ultrasonographic findings related to prognosis in canine transitional cell carcinoma. Veterinary Radiology and Ultrasound 1, 79–84.
Harris, A.L., Neal, D.E., 1992. Bladder cancer – Field versus clonal origin. The New England Journal of Medicine 326, 759–761.
Hautmann, R.E., 2008. [Ileal bladder substitute]. Der Urologe 47, 33–40.
Heng, H.G., Lowry, J.E., Boston, S., Gabel, C., Ehrhart, N., Gulden, S.M.S., 2006. Smooth muscle neoplasia of the urinary bladder wall in three dogs. Veterinary Radiology and Ultrasound 47, 83–86.
Henry, C.J., McCaw, D.L., Turnquist, S.E., Tyler, J.W., Bravo, L., Sheafor, S., Straw, R.C., Dernell, W.S., Madewell, B.R., Jorgensen, L., et al., 2003a. Clinical evaluation of mitoxantrone and piroxicam in a canine model of human invasive urinary bladder carcinoma. Clinical Cancer Research 9, 906–911.
Henry, C.J., Tyler, J.W., McEntee, M.C., Stokol, T., Rogers, K.S., Chun, R., Garrett, L.D., McCaw, D.L., Higginbotham, M.L., Flessland, K.A., et al., 2003b. Evaluation of a bladder tumor antigen test as a screening test for transitional cell carcinoma of the lower urinary tract in dogs. American Journal of Veterinary Research 64, 1017–1020.
Higuchi, T., Burcham, G.N., Childress, M.O., Rohleder, J.J., Bonney, P.L., Ramos-Vara, J.A., Knapp, D.W., 2013. Characterization and treatment of transitional cell carcinoma of the abdominal wall in dogs: 24 cases (1985–2010). Journal of the American Veterinary Medical Association 242, 499–506.
Holak, P., Nowicki, M., Adamiak, Z., Kasprowicz, A., 2007. Applicability of endoscopic examination as a diagnostic approach in urinary tract ailments in dogs. Polish Journal of Veterinary Sciences 10, 233–238.
Hume, C., Seiler, G., Porat-Mosenco, Y., Caceres, A., Shofer, F., Sorenmo, K., 2010. Cystosonographic measurements of canine bladder tumours. Veterinary and Comparative Oncology 8, 122–126.
Iida, N., Dzutsev, A., Stewart, C.A., Smith, L., Bouladoux, N., Weingarten, R.A., Molina, D.A., Salcedo, R., Back, T., Cramer, S., et al., 2013. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 342, 967–970.
Kessler, M., Kandel-Tschiederer, B., Pfleghaar, S., Tassani-Prell, M., 2008. Primary malignant lymphoma of the urinary bladder in a dog: Longterm remission following treatment with radiation and chemotherapy. Schweizer Archiv für Tierheilkunde 150, 565–569.
Knapp, D.W., 2006. Animal models; naturally occurring canine urinary bladder cancer. In: Lerner, S.P., Schoenberg, M.P., Sternberg, C.N. (Eds.), Textbook of Bladder Cancer, First Ed. The Taylor and Francis Group, Oxon, United Kingdom, pp. 171–175.
Knapp, D.W., McMillan, S.K., 2013. Tumors of the urinary system. In: Withrow, S.J., Vail, D.M. (Eds.), Withrow and MacEwen's Small Animal Clinical Oncology, Fifth Ed. Elsevier-Saunders, St. Louis, MO, USA, pp. 572–582.
Knapp, D.W., Glickman, N.W., Widmer, W.R., DeNicola, D.B., Adams, L.G., Kuczek, T., Bonney, P.L., DeGortari, A.E., Han, C., Glickman, L.T., 2000. Cisplatin versus cisplatin combined with piroxicam in a canine model of human invasive urinary bladder cancer. Cancer Chemotherapy and Pharmacology 46, 221–226.
Knapp, D.W., Peer, W.A., Conteh, A., Diggs, A.R., Cooper, B.R., Glickman, N.W., Bonney, P.L., Stewart, J.C., Glickman, L.T., Murphy, A.S., 2013a. Detection of herbicides in the urine of pet dogs following home lawn chemical application. The Science of the Total Environment 456–457, 34–41.
Knapp, D.W., Henry, C.J., Widmer, W.R., Tan, K.M., Moore, G.E., Ramos-Vara, J.A., Lucroy, M.D., Greenberg, C.B., Greene, S.N., Abbo, A.H., et al., 2013b. Randomized trial of cisplatin versus firocoxib versus cisplatin/firocoxib in dogs with transitional cell carcinoma of the urinary bladder. Journal of Veterinary Internal Medicine 27, 126–133.
Knapp, D.W., Ramos-Vara, J.A., Moore, G.E., Dhawan, D., Bonney, P.L., Young, K.E., 2014. Urinary bladder cancer in dogs, a naturally occurring model for cancer biology and drug development. ILAR Journal 55, 100–118.
Lai, C.-L., van den Ham, R., van Leenders, G., van der Lugt, J., Mol, J.A., Teske, E., 2008. Histopathological and immunohistochemical characterization of canine prostate cancer. The Prostate 68, 477–488.
Lin, T.Y., Zhang, H., Wang, S., Xie, L., Li, B., Rodriguez, C.O., Pan, C., 2011. Targeting canine bladder transitional cell carcinoma with a human bladder cancer-specific ligand. Molecular Cancer 10, 9.
Liptak, J., Dernell, W., Withrow, S., 2004a. Haemangiosarcoma of the urinary bladder in a dog. Australian Veterinary Journal 82, 215–217.
Liptak, J.M., Brutscher, S.P., Monnet, E., Dernell, W.S., Twedt, D.C., Kazmierski, K.J., Walter, C.U., Mullins, M.N., Larue, S.M., Withrow, S.J., 2004b. Transurethral resection in the management of urethral and prostatic neoplasia in 6 dogs. Veterinary Surgery 33, 505–516.
Lu, Z., Yeh, T.-K., Wang, J., Chen, L., Lyness, G., Xin, Y., Wienties, M.G., Bergdall, V., Couto, G., Alvarez-Berger, F., et al., 2011. Paclitaxel gelatin nanoparticles for intravesical bladder cancer therapy. The Journal of Urology 185, 1478–1483.
Marconato, L., Zini, E., Lindner, D., Suslak-Brown, L., Nelson, V., Jeglum, A.K., 2011. Toxic effects and antitumor response of gemcitabine in combination with piroxicam treatment in dogs with transitional cell carcinoma of the urinary bladder. Journal of the American Veterinary Medical Association 238, 1004–1010.
Martinez, I., Mattoon, J.S., Eaton, K.A., Chew, D.J., DiBartola, S.P., 2003. Polypoid cystitis in 17 dogs (1978–2001). Journal of Veterinary Internal Medicine 17, 499–509.
McMillan, S.K., Boria, P., Moore, G.E., Widmer, W.R., Bonney, P.L., Knapp, D.W., 2011. Antitumor effects of deracoxib treatment in 26 dogs with transitional cell carcinoma of the urinary bladder. Journal of the American Veterinary Medical Association 239, 1084–1089.
McMillan, S.K., Knapp, D.W., Ramos-Vara, J.A., Bonney, P.L., Adams, L.G., 2012. Outcome of urethral stent placement for management of urethral obstruction secondary to transitional cell carcinoma in dogs: 19 cases (2007–2010). Journal of the American Veterinary Medical Association 241, 1627–1632.
Messer, J.S., Chew, D.J., McLoughlin, M.A., 2005. Cystoscopy: Techniques and clinical applications. Clinical Techniques in Small Animal Practice 20, 52–64.

Mohammed, S.I., Craig, B.A., Mutsaers, A.J., Glickman, N.W., Snyder, P.W., deGortari, A.E., Knapp, D.W., 2003. Effects of the cyclooxygenase inhibitor, piroxicam, in combination with chemotherapy on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. Molecular Cancer Therapeutics 2, 183–188.

Moore, A.S., Cardona, A., Shapiro, W., Madewell, B.R., 1990. Cisplatin (cisdiamminedichloroplatinum) for treatment of transitional cell carcinoma of the urinary bladder or urethra. A retrospective study of 15 dogs. Journal of Veterinary Internal Medicine 4, 148–152.

Nam, E.-H., Ko, S., Chae, J.-S., Hwang, C.-Y., 2013. Characterization and zoonotic potential of uropathogenic Escherichia coli isolated from dogs. Journal of Microbiology and Biotechnology 23, 422–429.

Naughton, J.F., Widmer, W.R., Constable, P.D., Knapp, D.W., 2012. Accuracy of three-dimensional and two-dimensional ultrasonography for measurement of tumor volume in dogs with transitional cell carcinoma of the urinary bladder. American Journal of Veterinary Research 73, 1919–1924.

Nieset, J.R., Harmon, J.F., Johnson, T.E., Larue, S.M., 2014. Comparison of adaptive radiotherapy techniques for external radiation therapy of canine bladder cancer. Veterinary Radiology and Ultrasound 55, 644–650.

Nolan, M.W., Kogan, L., Griffin, L.R., Custis, J.T., Harmon, J.F., Biller, B.J., Larue, S.M., 2012. Intensity-modulated and image-guided radiation therapy for treatment of genitourinary carcinomas in dogs. Journal of Veterinary Internal Medicine 4, 987–995.

Nyland, T.G., Wallack, S.T., Wisner, E.R., 2002. Needle-tract implantation following us-guided fine-needle aspiration biopsy of transitional cell carcinoma of the bladder, urethra, and prostate. Veterinary Radiology and Ultrasound 43, 50–53.

Ohnishi, S., Ma, N., Thanan, R., Pinlaor, S., Hammam, O., Murata, M., Kawanishi, S., 2013. DNA damage in inflammation-related carcinogenesis and cancer stem cells. Oxidative Medicine and Cellular Longevity 2013, 387014.

Owen, L.N., World Health Organization, 1980. TNM Classification of Tumours in Domestic Animals. World Health Organization (WHO), Geneva.

Pancotto, T.E., Rossmeisl, J.H., Zimmerman, K., Robertson, J.L., Werre, S.R., 2013. Intramedullary spinal cord neoplasia in 53 dogs (1990–2010): Distribution, clinicopathologic characteristics, and clinical behavior. Journal of Veterinary Internal Medicine 27, 1500–1508.

Patrick, D.J., Fitzgerald, S.D., Sesterhenn, I.A., Davis, C.J., Kiupel, M., 2006. Classification of canine urinary bladder urothelial tumours based on the World Health Organization/International Society of Urological Pathology consensus classification. Journal of Comparative Pathology 135, 190–199.

Poirier, V.J., Forrest, L.J., Adams, W.M., Vail, D.M., 2004. Piroxicam, mitoxantrone, and coarse fraction radiotherapy for the treatment of transitional cell carcinoma of the bladder in 10 dogs: A pilot study. Journal of the American Animal Hospital Association 40, 131–136.

Raghavan, M., Knapp, D.W., Dawson, M.H., Bonney, P.L., Glickman, L.T., 2004. Topical flea and tick pesticides and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. Journal of the American Veterinary Medical Association 225, 389–394.

Raghavan, M., Knapp, D.W., Bonney, P.L., Dawson, M.H., Glickman, L.T., 2005. Evaluation of the effect of dietary vegetable consumption on reducing risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. Journal of the American Veterinary Medical Association 227, 94–100.

Ramos-Vara, J.A., Miller, M.A., Boucher, M., Roudabush, A., Johnson, G.C., 2003. Immunohistochemical detection of uroplakin III, cytokeratin 7, and cytokeratin 20 in canine urothelial tumors. Veterinary Pathology 40, 55–62.

Reed, L.T., Knapp, D.W., Miller, M.A., 2013. Cutaneous metastasis of transitional cell carcinoma in 12 dogs. Veterinary Pathology 50, 676–681.

Robat, C., Burton, J., Thamm, D., Vail, D., 2013. Retrospective evaluation of doxorubicin-piroxicam combination for the treatment of transitional cell carcinoma in dogs. The Journal of Small Animal Practice 54, 67–74.

Salinardi, B.J., Marks, S.L., Davidson, J.R., Senior, D.F., 2003. The use of a low-profile cystostomy tube to relieve urethral obstruction in a dog. Journal of the American Animal Hospital Association 39, 403–405.

Saulnier-Troff, F.-G., Busoni, V., Hamaide, A., 2008. A technique for resection of invasive tumors involving the trigone area of the bladder in dogs: Preliminary results in two dogs. Veterinary Surgery 37, 427–437.

Schrempp, D.R., Childress, M.O., Stewart, J.C., Leach, T.N., Tan, K.M., Abbo, A.H., Knapp, D.W., 2013. Metronomic administration of chlorambucil for treatment of dogs with urinary bladder transitional cell carcinoma. Journal of the American Veterinary Medical Association 242, 1534–1538.

Sledge, D.G., Patrick, D.J., Fitzgerald, S.D., Xie, Y., Kiupel, M., 2014. Differences in expression of uroplakin III, cytokeratin 7, and cyclooxygenase-2 in canine proliferative urothelial lesions of the urinary bladder. Veterinary Pathology doi:10.1177/0300985814522819.

Smith, J.D., Stone, E.A., Gilson, S.D., 1995. Placement of a permanent cystostomy catheter to relieve urine outflow obstruction in dogs with transitional cell carcinoma. Journal of the American Veterinary Medical Association 206, 496–499.

Stone, E.A., Withrow, S.J., Page, R.L., Schwarz, P.D., Wheeler, S.L., Seim, H.B., 1988. Ureterocolonic anastomosis in ten dogs with transitional cell carcinoma. Veterinary Surgery 17, 147–153.

Stratmann, N., Wehrend, A., 2007. Unilateral ovariectomy and cystectomy due to multiple ovarian cysts with subsequent pregnancy in a Belgian shepherd dog. The Veterinary Record 160, 740–741.

Thompson, M.F., Litster, A.L., Platell, J.L., Trott, D.J., 2011. Canine bacterial urinary tract infections: New developments in old pathogens. The Veterinary Journal 190, 22–27.

Valli, V.E., Norris, A., Jacobs, R.M., Laing, E., Withrow, S., Macy, D., Tomlinson, J., McCaw, D., Ogilvie, G.K., Pidgeon, G., et al., 1995. Pathology of canine bladder and urethral cancer and correlation with tumour progression and survival. Journal of Comparative Pathology 113, 113–130.

Vignoli, M., Rossi, F., Chierici, C., Terragni, R., De Lorenzi, D., Stanga, M., Olivero, D., 2007. Needle tract implantation after fine needle aspiration biopsy (FNAB) of transitional cell carcinoma of the urinary bladder and adenocarcinoma of the lung. Schweizer Archiv für Tierheilkunde 149, 314–318.

Wagner, S., Gally, D.L., Argyle, S.A., 2014. Multidrug-resistant Escherichia coli from canine urinary tract infections tend to have commensal phylotypes, lower prevalence of virulence determinants and ampC-replicons. Veterinary Microbiology 169, 171–178.

Walker, M., Breider, M., 1987. Intraoperative radiotherapy of canine bladder cancer. Veterinary Radiology 28, 200–204.

Weisse, C., Berent, A., Todd, K., Clifford, C., Solomon, J., 2006. Evaluation of palliative stenting for management of malignant urethral obstructions in dogs. Journal of the American Veterinary Medical Association 229, 226–234.

Withrow, S.J., Gillette, E.L., Hoopes, P.J., McChesney, S.L., 1989. Intraoperative irradiation of 16 spontaneously occurring canine neoplasms. Veterinary Surgery 18, 7–11.

Wongsetthachai, P., Pramatwinai, C., Banlunara, W., Kalpravidh, M., 2011. Urinary bladder wall substitution using autologous tunica vaginalis in male dogs. Research in Veterinary Science 90, 156–159.

Zhang, H., Aina, O.H., Lam, K.S., de Vere White, R., Evans, C., Henderson, P., Pan, C.-X., 2012. Identification of a bladder cancer-specific ligand using a combinatorial chemistry approach. Urologic Oncology 30, 635–645.

Zhang, Y., Frimberger, D., Cheng, E.Y., Lin, H.-K., Kropp, B.P., 2006. Challenges in a larger bladder replacement with cell-seeded and unseeded small intestinal submucosa grafts in a subtotal cystectomy model. BJU International 98, 1100–1105.