Urinary Bladder Cancer in Dogs, a Naturally Occurring Model for Cancer Biology and Drug Development

Deborah W. Knapp, José A. Ramos-Vara, George E. Moore, Deepika Dhawan, Patty L. Bonney, and Kirsten E. Young

Abstract

Each year more than 65,000 people are diagnosed with urinary bladder cancer, and more than 14,000 people die from the disease in the United States. Studies in relevant animal models are essential to improve the management of bladder cancer. Naturally occurring bladder cancer in dogs very closely mimics human invasive bladder cancer, specifically high-grade invasive transitional cell carcinoma (TCC; also referred to as invasive urothelial carcinoma) in cellular and molecular features; biological behavior, including sites and frequency of metastasis; and response to therapy. Canine bladder cancer complements experimentally induced rodent tumors in regard to animal models of bladder cancer. Results of cellular and molecular studies and -omics analyses in dogs are expected to lead to improved detection of TCC and preneoplastic lesions, earlier intervention, better prediction of patient outcome, and more effective TCC management overall. Studies in dogs are being used to help define heritable risks (through very strong breed-associated risk) and environment risks and to evaluate prevention and treatment approaches that benefit humans as well as dogs. Clinical treatment trials in pet dogs with TCC are considered a win–win scenario by clinician scientists and pet owners. The individual dog benefits from effective treatment, the results are expected to help other dogs, and the findings are expected to ultimately help humans with TCC. This article provides an overview of canine TCC, a summary of the similarities and differences between canine and human invasive TCC, and examples of the types of valuable translational research that can be done using dogs with naturally occurring TCC.

Key Words: animal model; bladder cancer; dog; transitional cell carcinoma

The Human Bladder Cancer Problem and Need for Animal Models

Each year more than 65,000 people are diagnosed with urinary bladder cancer, and more than 14,000 people die from this cancer in the United States (Burger et al. 2013; Lerner et al. 2006; Tanaka and Sonpavde 2011). Worldwide more than 350,000 cases are expected to occur yearly (Ploeg et al. 2009). Transition cell carcinoma (TCC), also referred to as urothelial carcinoma, comprises the vast majority of bladder cancer. It occurs in two broad forms, low-grade, superficial disease and high-grade, invasive cancer. In humans, more than two-thirds of bladder tumors at diagnosis are superficial low-grade TCCs confined to the bladder mucosa (Cheng et al. 2012; Lerner et al. 2006). These tumors generally respond well to transurethral resection and intravesical therapy, although recurrence is common, quality of life is negatively affected, and progression to invasive TCC is a risk. Approximately 20% of human bladder cancers are higher-grade invasive TCC at the time of diagnosis. The standard treatment for human invasive TCC is cystectomy to address the primary tumor and chemotherapy for metastases. Radiation therapy is used in bladder-sparing procedures and for regional metastases. Metastasis to regional lymph nodes, lungs, and other organs occurs in approximately 50% of people with invasive TCC (Lerner et al. 2006). Most bladder cancer deaths are due to metastases. There is clearly a great need to improve the outlook for people with TCC, and research involving animal models of TCC is essential.

Several experimentally induced models of TCC have been established, including chemically induced tumors, transgenic mouse models, and orthotopic xenograft models (Arantes-Rodrigues et al. 2013; Wu et al. 2006). Although these

Deborah W. Knapp, MS, DVM, is a professor of comparative oncology and a director of the Purdue Comparative Oncology Program at the Purdue University College of Veterinary Medicine and a co-program leader in the Purdue University Center for Cancer Research in West Lafayette, Indiana. José A. Ramos-Vara, DVM, PhD, is a professor of comparative pathobiology at the Purdue University College of Veterinary Medicine in West Lafayette, Indiana. George E. Moore, DVM, PhD, is a professor of epidemiology and director of the Clinical Trials Program at the Purdue University College of Veterinary Medicine in West Lafayette, Indiana. Deepika Dhawan, PhD, is a research scientist in cancer biology at the Purdue University College of Veterinary Medicine in West Lafayette, Indiana. Patty L. Bonney, BS, RVT, is a clinical trials coordinator and program associate at the Purdue University College of Veterinary Medicine in West Lafayette, Indiana. Kirsten E. Young, BS, has previously worked in the Knapp laboratory and is currently a veterinary student at the Purdue University College of Veterinary Medicine in West Lafayette, Indiana.

Address correspondence and reprint requests to Dr. Deborah W. Knapp, Purdue University, Department of Veterinary Clinical Sciences, 625 Harrison Street, West Lafayette, IN 47907-2026 or email knappd@purdue.edu.

ILAR Journal, Volume 55, Number 1, doi: 10.1093/ilar/ilu018

experimental animal models are of great value and are constantly in use in bladder cancer research, there is still a need for complementary animal models in which the disease is naturally occurring, invades and metastasizes consistently, and more closely mimics the human condition. Dogs with naturally occurring invasive TCC provide an ideal animal model to address this need (Knapp 2006). The majority of canine TCC consists of high-grade invasive cancer, and this will be the focus of this review. The purpose of this article is to provide an overview of canine TCC, to summarize the similarities between canine and human invasive TCC, and to discuss the types of valuable translational research that can be done using dogs with naturally occurring TCC.

Naturally Occurring Bladder Cancer in Dogs

Bladder cancer comprises approximately 2% of all naturally occurring cancers in dogs, similar to rates in humans (Knapp and McMillan 2013; Lerner et al. 2006). With more than 70 million pet dogs in the United States and cancer developing in approximately 25% of older dogs, it is expected that bladder cancer will newly affect more than 20,000 dogs each year. The diagnosis is made by histologic exam of tissue biopsies collected by surgery, cystoscopy, or catheter biopsy (Knapp and McMillan 2013). The vast majority of TCC in dogs (> 90% of cases) consists of intermediate- to high-grade invasive TCC (Patrick et al. 2006; Valli et al. 1995). Superficial, low-grade TCC is very uncommon in dogs.

Risk factors for TCC in dogs include female sex, history of spay or neuter, obesity, strong breed-related risk (discussed in more detail herein), and exposure to older generation flea control products and lawn chemicals (Glickman et al. 1989; Glickman et al. 2004; Knapp and McMillan 2013). The female / male ratio of dogs with TCC has been reported to range from 1.71:1 to 1.95:1, with risk further enhanced in spayed or neutered dogs (Bryan et al. 2007; Knapp and McMillan 2013). Vegetable consumption has been reported to reduce the risk of TCC in Scottish terriers who have a strong breed-associated risk for the disease (Raghavan et al. 2005).

TCC typically occurs in older dogs with reported mean and median ages at diagnosis ranging from 9 to 11 years, although the disease can occur much earlier in a minority of dogs (Knapp and McMillan 2013). TCC is most often located in the trigone region of the bladder. Papillary lesions and a thickened bladder wall can lead to partial or complete urinary tract obstruction. TCC has also been reported to involve the urethra in 56% of dogs and the prostate in 29% of male dogs (Knapp, Glickman, Denicola, et al. 2000). Nodal and distant metastases have been reported in approximately 16% of dogs at diagnosis and 50% of dogs at death (Knapp, Glickman, Denicola, et al. 2000). When applying World Health Organization (WHO) criteria for staging canine bladder tumors (Owen 1980) (Table 1), 78% of dogs have been reported to have T2 tumors and 20% of dogs to have T3 tumors (Knapp, Glickman, Denicola, et al. 2000). There is a difference in the TNM staging system between dogs and humans, with T2 tumors in dogs including muscle invasive disease, whereas muscle invasive tumors in humans are classified as T3 or greater.

Table 1 World Health Organization TNM clinical staging system for canine bladder cancer

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

Modified from Owen LN. 1980. TNM Classification of Tumours in Domestic Animals. Geneva: World Health Organization.

The treatment of TCC in dogs can include surgery, radiation therapy, chemotherapy and other drugs, or combinations of these, although surgery and radiation therapy are used less often than drug therapy in dogs (Knapp and McMillan 2013). Complete cystectomy, which is the front-line treatment for organ-confined invasive bladder cancer in humans, is not typically performed in pet dogs because of the frequent presence of cancer beyond the bladder (urethra, prostate, other organs), the morbidity of the procedure, and the expense involved (Knapp and McMillan 2013; Vilar et al. 2004). Most TCC lesions in dogs are not in a location where complete surgical excision is possible. Initial reports of radiation therapy in dogs with TCC were discouraging because of the side effects of the treatment (Anderson et al. 2002). In recent reports, however, newer radiation therapy approaches have been much better tolerated, allowing further study (Nolan et al. 2012; Poirier et al. 2004).

Chemotherapy, cyclooxygenase inhibitors, and combinations of these are the mainstay of treatment for primary and metastatic TCC in dogs (Knapp and McMillan 2013). Remission rates are typically less than 20% with single-agent therapy, and 35–50% with combined chemotherapy and cox inhibitor treatment. Platinum agents appear to be the most active in canine TCC, especially when combined with a cox inhibitor (Knapp and McMillan 2013; Knapp, Henry, et al. 2013). Chemotherapy can be delivered in dogs with minimal risk of bothersome side effects and with maintained or improved quality of life. Other important components of the

care of dogs with TCC include the placement of urethral and ureteral stents when needed to relieve urinary obstruction and aggressively treating the increasingly resistant secondary bacterial infections (Blackburn et al. 2013; Childress et al. 2011; Smee et al. 2013). Although TCC is not usually curable in dogs with current therapies, the disease can be controlled in 75% of dogs, and the dogs can enjoy many months to a year or more of good-quality life after diagnosis. The most consistent prognostic factor is TNM stage, with more advanced stage being associated with shorter survival. Across dogs with all stages of TCC, median survival times are typically between 130 and 195 days after single-agent drug treatment and more than 250 days after combination drug treatments (Knapp and McMillan 2013). When multiple treatments are given sequentially, survival can extend well beyond a year. Thus, even though TCC is rarely curable, it is considered highly treatable.

Treatment studies in dogs are expected to be a win–win–win scenario. The individual dog receives treatment that is expected to help them and that often provides hope when other treatments are not effective or not feasible. The results are expected to help other dogs. And the results are expected to ultimately help humans with TCC. Many treatment trials for pet dogs also include funding from the trial sponsor, which enables pet owners to pursue treatment for their dog even if they cannot afford other therapies. In fact, at the Purdue University College of Veterinary Medicine, where the authors work, more than 90% of owners of dogs with TCC elect to enroll their pet in a clinical trial. Parallel mechanism studies are feasible in dogs with samples of blood, urine, and, in some cases, tumor tissues collected by cystoscopy available before and during therapy. Most pet owners will also allow a necropsy of the dog when it dies or is euthanized (because of declining quality of life from cancer progression or other conditions). This provides crucial information on the disease process and response to therapy and the opportunity to bank tissue samples for future studies. Although most treatments tested in dogs have been systemic therapies, dog studies can also be used to evaluate intravesical therapy (Abbo et al. 2010).

Similarities and Differences between Bladder Cancer in Humans and Dogs

Similarities in the Pathology and Metastatic Behavior

The microscopic anatomy of the canine urinary bladder is similar to that of the human bladder. TCC, also referred to as urothelial carcinoma, is the most commonly reported tumor in the urinary bladder of domestic animals, comprising approximately 2% of all malignant neoplasms in dogs (Meuten and Meuten 2014). By growth pattern, TCCs are divided into papillary (50%) or nonpapillary (50%) tumors and infiltrating (≥90%) or noninfiltrating (≤10%) tumors (Meuten and Meuten 2014). Although noninfiltrating tumors comprise the majority of human TCCs (≥65% of cases), this form of bladder cancer is uncommon in dogs. The genesis of bladder cancer is thought to arise through alterations in two distinct molecular pathways. Low-grade, noninvasive TCCs usually have alterations in the Ras–mitogen-activated protein kinase signal transduction pathway, whereas invasive tumors have deregulations in the p53 and retinoblastoma pathways (Mitra et al. 2012; Gamblin et al. 1997; Mitra et al. 2006; Mitra et al. 2012). It is likely that activation of pathways leading to low-grade tumors is uncommon in dogs. Great similarity, however, is noted in the microscopic features between invasive TCC in dogs and humans (Knapp, Glickman, Denicola, et al. 2000). Studies of canine TCC are expected to be of the most translational value in the invasive form of the disease. With the relatively late diagnosis of TCC in dogs, carcinoma in situ, an accepted precursor to invasive TCC (Cheng et al. 2010), is uncommonly observed in dogs (Meuten and Meuten 2014). Cytoplasmic cystic degeneration involving single or multiple urothelial cells is common in canine TCC. Although not unique to TCC, this feature aids in diagnosis of TCC in metastatic disease of unknown primary neoplasm (Meuten and Meuten 2014). Another common feature of canine TCC is a marked desmoplastic reaction to invasion in primary and metastatic lesions. Infiltrates of leukocytes are also common in primary tumors in dogs and humans. One difference noted between canine and human invasive TCC is the location within the bladder. The majority of canine TCC is trigonal in location at diagnosis, whereas there is more variation in initial tumor location within the bladder in humans. (Meuten and Meuten 2014; Mutsaers et al. 2003). Distant metastases have been reported in 15–20% of dogs at diagnosis and in at least 50% of dogs at death (Knapp and McMillan 2013; Meuten and Meuten 2014). A near-infrared imaging approach to identify sentinel lymph nodes at the time of surgery has been investigated in dogs (Knapp et al. 2007). Metastatic disease is more common in anaplastic tumors, followed by nonpapillary and infiltrating variants (Meuten and Meuten 2014). Lungs and regional lymph nodes are the most common reported sites of metastatic disease, but other organs, including the skin, can be affected (Reed et al. 2013).

Necropsy findings have recently been compiled from 137 dogs with TCC evaluated at Purdue University to further characterize the metastatic disease pattern (Table 2). Of the 137 dogs, 92 dogs (67%) had metastases in at least one site. Nodal metastases alone (in absence of distant metastases) were found in 9% of dogs, distant metastases alone were found in 25% of dogs, and a combination of nodal and distant metastases were identified in 33% of dogs at the time of death. The frequency of metastasis and the sites involved were similar between dogs and humans (Table 2), with lung being the most common site of distant metastasis. In addition to visceral and nodal metastasis, TCC also spreads to the abdominal wall through instruments and needles used in surgical and nonsurgical procedures and naturally along ligaments that support the bladder (Higuchi et al. 2013). In this location, the cancer typically grows aggressively and is poorly responsive to medical therapy. Bone metastases are

Table 2 Sites of metastases identified in 137 dogs with transitional cell carcinoma undergoing necropsy at Purdue University between 2005 and 2013 with comparison with published autopsy findings from humans (Wallmeroth et al. 1999)

Location of metastases	Number of dogs with metastasis in that location (% of 137 dogs undergoing necropsy)	Number of humans with metastases in that location (% of 308 humans undergoing autopsy)
Any metastases	92 (67)	214 (69)
Any nodal metastases	57 (42)	180 (58)
Regional nodes (abdominal, pelvic inguinal nodes)[a]	40 (29)	158 (51)
Thoracic nodes[b]	17 (12)	80 (26)
Other nodes	1 (1)	8 (3)
Any distant metastases	80 (58)	147 (48)
Lung	69 (50)	96 (31)
Bone	15 (11)	71 (23)
Liver	10 (7)	103 (33)
Kidney[c]	10 (7)	30 (10)
Adrenal gland	8 (6)	28 (10)
Skin	8 (6)	4 (1.5)
Spleen	6 (4)	11 (3.6)
Gastrointestinal[d]	3 (2)	45 (15)
Heart	5 (4)	13 (4)
Brain	2 (1.5)	8 (2.5)

[a]Nodes included 32 iliac, sacral, and other sublumbar; 3 inguinal; 2 mesenteric; 2 pancreatic; and 1 hypogastric node.
[b]Nodes included nine tracheobronchial, four sternal, three mediastinal, and one hilar node
[c]It was not always possible to determine if the transitional cell carcinoma represented a second primary site in the kidney or a metastatic lesion.
[d]Tumor location included stomach in one dog, jejunum in one dog, and pancreas in one dog.

also emerging as an important metastatic site, although further prospective study is needed to determine the frequency and characteristics of these lesions. An additional interesting finding from the necropsy study was the presence of second primary tumors in 18 of the 137 dogs (13%) with TCC. Second primary tumors included hemangiosarcoma (n = 3), marginal zone lymphoma (n = 3), hepatocholangio carcinoma (n = 2), follicular thyroid carcinoma (n = 2), B cell lymphoma (n = 2), adrenal adenocarcinoma (n = 1), meningioma (n = 1), nasal adenocarcinoma (n = 1), cutaneous squamous cell carcinoma (n = 1), oral melanoma (n = 1), pancreatic adenocarcinoma (n = 1), undifferentiated neuroendocrine tumor (n = 1), histiocytic sarcoma (n = 1), and splenic sarcoma (n = 1). In an autopsy study of 376 humans with invasive bladder cancer, second primary tumors were found in 74 (20%) patients, with most being carcinomas arising from other primary sites.

Several classification / grading schemes have been published in veterinary medicine for canine urothelial neoplasms, particularly TCC. One classification (Valli et al. 1995) is based on the WHO scheme modified by Mostofi and colleagues (1986); another classification scheme (Patrick et al. 2006) is based on a more recent World Health Organization/International Society of Urological Pathology (WHO/ISUP) classification (Epstein et al. 1998). The WHO/ISUP consensus classification of urothelial neoplasms (Epstein et al. 1998) classifies urothelial lesions as hyperplasia, flat lesions with atypia, and papillary neoplasms. Based on this classification, Patrick and colleagues (2006) reported that the majority of canine tumors examined were grade II (intermediate grade) or grade III (high grade) papillary carcinomas. Other authors reported similar results (Valli et al. 1995). Valli and colleagues (1995) used a modified version by Mostofi and colleagues (1986) of the then-current WHO classification. This classification scheme incorporates multiple gross and microscopic features of bladder lesions (e.g., histology, growth pattern, depth of the tumor invasion, nuclear appearance, vascular invasion) to classify and grade urinary bladder tumors. Features associated with localized disease in canine urothelial carcinoma included papillary architecture, in situ tumor, low tumor grade, and peri-tumoral lymphoid cell reaction, whereas typical features in dogs with metastatic disease included infiltrating and nonpapillary architecture, increasing tumor grade, depth of invasion, vascular invasion, and presence of peri-tumoral fibrosing reaction. Shorter survival was observed with grade II and III tumors when compared with grade I tumors (Valli et al. 1995).

When surgical specimens are examined, there is usually sufficient tissue to assess growth pattern, depth of invasion, vascular invasion, and so on. When tissue biopsies are obtained by cystoscopy, however, the size of the biopsies is often small. In our opinion, nuclear grade is one of the few constant features that can be evaluated in tissues, including small tissue samples. In our institution, using nuclear grade as the only feature evaluated in a retrospective study of 232 TCCs (biopsies or postmortem specimens), two TCCs were grade I; 67 TCCs were were grade II; and 163 TCCs were grade III (J. Ramos-Vara, unpublished data). The authors of a recent publication (Cheng et al. 2012) question the value of including papillary urothelial neoplasms of low malignant potential in the current WHO/ISUP classification of human urothelial neoplasms (Epstein 2010) and recommend incorporation of these tumors with the grade I (low grade) urothelial carcinoma. The new proposed grading scheme for human TCC is four tiered (grades I–IV, with grade IV being the most malignant) (Cheng et al. 2012). This grading system appears to be of limited value in canine TCC because the large majority of TCC in dogs is of a high grade. Because of this fact and the interobserver variability in application of a multitier system for TCC and other tumors (Kiupel et al. 2011; Meuten and Meuten 2014), we propose the use of a two-tier simplified grading scheme (low vs. high grade) based on the nuclear atypia and organization of cells (Cheng et al. 2012; Epstein 2010). Low-grade TCC has minimal or focal cellular atypia, with rare or infrequent mitotic figures that can be present at any level of the urothelium. High-grade TCC includes those lesions with obvious architectural disorder, nuclear atypia, and increased number of mitotic figures. Using the two-tier system in 232 TCCs, six cases were considered of low grade and 226 were considered of high grade (J. Ramos-Vara, unpublished data).

The microscopic diagnosis of TCC is typically straightforward when adequate tissue is available for examination. Obtaining sufficient tissue samples by cystoscopy can be a challenge, and the operator must be diligent to collect enough tissue for diagnosis. In a report of 92 dogs, diagnostic samples were obtained by cystoscopy in 96% of female dogs and 65% of male dogs (in which smaller scopes are required) that ultimately had hisotopathologically diagnosed TCC (Childress et al. 2011). Placing tissue samples in a histology cassette before processing to prevent loss of small samples and using a wire stone collection basket to obtain larger pieces of tissue during cystoscopy has further improved the yield of diagnostic biopsy samples.

Immunohistochemistry is useful for small biopsy specimens of bladder masses, for tumors located outside the urinary tract, and to confirm histogenesis of individual or small groups of cells in the bladder. The most common marker of urothelial differentiation used in dogs is uroplakin III (Ramos-Vara et al. 2003). However, as in human urothelial carcinoma, uroplakin III, although highly specific for normal urothelium and its tumors, has relatively low sensitivity, which can be a problem when evaluating small (cystoscopic) samples. Work is in progress to evaluate other markers currently used for human TCC such as UPII, GATA-3, p63, and placental S100 (Carvalho et al. 2012; Higgins et al. 2007; Langner et al. 2003; Miyamoto, Izumi, et al. 2012; Wu et al. 2005; Wu et al. 2009). Initial results (Figure 1) suggest these markers label canine TCC; further validation studies are needed to determine their sensitivity and specificity in the dog.

Other Cellular and Molecular Features

In addition to similarities in the pathologic findings between dogs and humans with invasive TCC, there is evidence for similarities in other cellular and molecular features studied to date (Knapp and McMillan 2013). Genomic, cytogenetic, proteomic, metabolomic, and lipidomic analyses are ongoing with initial findings published (Dill et al. 2009; Wilson et al. 2008; Zhang J et al. 2012). The expectation is -omics analyses will subsequently lead to new strategies to improve detection of TCC and preneoplastic lesions, intervene earlier in the course of disease, predict individual patient outcome, and more effectively manage the cancer overall. For example, understanding changes on the surface of epithelial cells that occur in the transition from normal to dysplasia to carcinoma in situ could allow the development of better assays to detect early changes in the cancer process. -Omics analyses are also expected to lead to the identification of new targets for therapy that have not yet been exploited in bladder cancer. Evidence for this comes from a recent report on an integrated genomic analysis that included whole-exome sequencing of 130 human TCC samples and matched normal samples (Cancer Genome Atlas Research Network 2014). The investigators reported that the analyses identified potential therapeutic targets in 69% of the tumors and included targets that had not previously attracted attention in TCC treatment approaches.

One of the best examples of similarities in -omics analyses between dogs and humans involves lipidomic profiling. Dill and colleagues (2009) have applied imaging mass spectrometry methods that combine chemical information collected for multiple analytes with spatial information in the analysis of histological tissue sections including TCC (Dill et al. 2009). Specifically, Dill and colleagues have applied desorption electrospray ionization and a microprobe imaging procedure that involves moving the probe spray continuously across the surface of the tissue section while recording mass spectra under ambient conditions without chemical pretreatment. Different lipid patterns involving glycerophospholipids, free fatty acids, and other lipid species were found between canine TCC and normal bladder sections. After the successful proof-of-concept work in canine TCC, similar methods were then applied to human TCC sections with similarities in the spectra noted between dogs and humans (Dill et al. 2011).

There are other examples of similarities between human and canine TCC. Cyclooxyegnease-2 (cox-2) is overexpressed in both canine and human bladder cancer, with

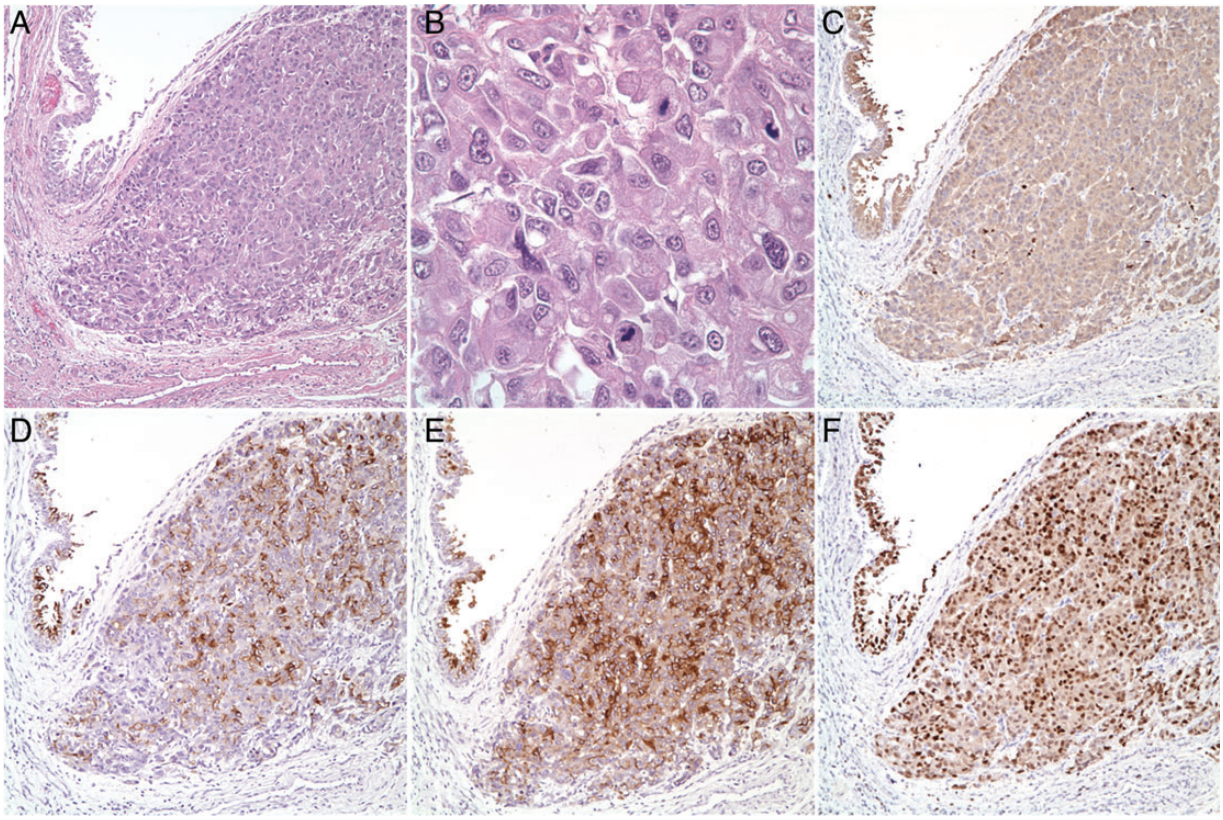

Figure 1 High-grade, invasive transitional cell carcinoma (urothelial carcinoma), urinary bladder, dog. (A) Invasion of the lamina propria by a disorganized mass of atypical urothelial epithelium. The overlying urothelium is eroded. Hematoxylin and eosin (H&E). (B) Detail of the neoplastic growth. Marked anisocytosis and anisokaryosis are observed. Note several mitotic figures. H&E. (C–F) Immunoperoxidase-3,3′-diaminobenzidine for placental S100 (C), uroplakin III (D), uroplakin II (E), and GATA-3 (F).

expression noted in invasive TCC and carcinoma in situ but not in the normal urothelium (Cekanova et al. 2013; Mohammed et al. 1999; Shirahama 2000; Wadhwa et al. 2005). Cox inhibitors have become an integral part of TCC treatment in dogs and have led to studies in humans with invasive TCC (Dhawan et al. 2010; Knapp and McMillan 2013). In another example, the expression of the apoptosis-inhibiting protein survivin has been found to be similar between dogs and humans. Survivin expression was reported in 359 of 726 human TCC cases (49%) and was negatively associated with prognosis (Shariat et al. 2009). Survivin has been targeted for urine tests to detect and monitor TCC (Ku et al. 2012; Swana et al. 1999). In dogs, survivin (specifically survivin localized to the nucleus) was detected by immunohistochemistry in 28 of 41 TCC tissues (68%) and in 0 of 46 normal bladder tissues, with findings confirmed by polymerase chain reaction (Rankin et al. 2008). Another example pertains to telomerase and its effects on telomeres. Telomeres form the ends of chromosomes, and along with the associated protein complex shelterin, protect the ends of the chromosomes (Orlando et al. 2001; Willeit et al. 2010). Telomeres progressively shorten by 30 to 200 base pairs with each division, and eventually the shortening of the telomeres results in initiation of cell senescence and apoptosis. Telomere maintenance is an important mechanism by which tumor cells evade senescence, and in most instances, it is accomplished by upregulating telomerase activity, which prevents telomere shortening. Telomerase activity has been detected in 90% of human TCCs, and it has been targeted in TCC detection assays (Chen and Chen 2011; Eissa et al. 2013; Orlando et al. 2001). Telomerase activity has been reported in a canine TCC cell line and in urine samples from dogs with TCC (McCleary-Wheeler et al. 2010).

Similarities in Clinical Presentation and Response to Therapy

TCC is generally a disease of older people and dogs. The median age at diagnosis of 102 dogs with TCC was 11 years (Knapp, Glickman, Denicola, et al. 2000). When applying formulas to convert the age in dogs to human equivalent years (Patronek et al. 1997), the median age at diagnosis was 60 years. In humans, the median age at diagnosis has been reported to range from the early 60s to the early 70s (Lerner et al. 2006).

Presenting clinical signs are also similar between dogs and humans, with hematuria being the most common change noted in both species. Initially, there is an absence of pain or urgency. As the cancer progresses, more frequent urination and stranguria develop. A history of urinary tract infections is

common in TCC cases in both species. The clinical signs may improve with antibiotics to treat the secondary infection but may not resolve completely with antibiotic treatment or may recur soon after the course of antibiotics. Although infrequent, pain associated with bone metastases occurs in dogs and humans.

There are similarities in the response to medical therapy in dogs and humans with TCC. Platinum agents are considered the most active agents in both species (Knapp and McMillan 2013; Lei et al. 2011). Cisplatin-based combination chemotherapy protocols are not often used in dogs because of side effects considered unacceptable in dogs, although a comparison of single-agent activity between dogs and humans is possible. The remission rate with single-agent cisplatin is 12–20% in dogs and 17–34% in humans (Ismaili et al. 2011; Knapp, Glickman, Widmer, et al. 2000; Miller and Torti 1992; Yagoda 1989). Carboplatin has activity in both species, although less than cisplatin (Boria et al. 2005; Ismaili et al. 2011). The previous standard protocol for TCC treatment in humans was methotrexate, vinblastine, doxorubicin, and cisplatin (Ismaili et al. 2011). Although this protocol was considered too toxic for acceptable use in pet dogs, an important component of the protocol, vinblastine, has been evaluated in dogs with remission and stable disease rates of 36% and 50%, respectively (Arnold et al. 2011). Vinblastine has single-agent activity in humans and contributes to combination therapy protocols (Blumenreich et al. 1982; Roberts et al. 2006). There is also evidence for activity of gemcitabine in both species (de Brito Galvao et al. 2012; Haggag et al. 2013).

Differences Between Bladder Cancer in Dogs and Bladder Cancer in Humans

TCC is very similar between dogs and humans, although there are at least three differences between the species. First, low-grade, superficial TCC comprises the majority of TCC in humans but is very uncommon in dogs. It is thought that the specific pathways that lead to low-grade, superficial TCC in humans are not active in dogs. In most instances, dog studies are of the greatest value in the study of invasive TCC. Second, the location of the TCC within the bladder can be different between dogs and humans. The majority of dogs have trigonal disease, and extension down the urethra is common (Knapp and McMillan 2013). In humans, however, there is a more balanced distribution of the TCC across the areas of the bladder (Meuten and Meuten 2014). The reason for this difference in distribution is not yet known. Third, there is a notable difference in sex predilection for TCC between dogs and humans. In humans, there is a 2:1 ratio for the occurrence in men versus women (Burger et al. 2013). In dogs, the cancer is less common in male dogs, with a 2:1 ratio in favor of female dogs (Bryan et al. 2007; Knapp and McMillan 2013). The sex difference in TCC risk is only partially understood. Over several decades, men have smoked more than women, and smoking is thought to be a causative factor for up to 50% of human TCC cases (Burger et al. 2013). Men have traditionally had more occupational exposures to chemicals. It is important to note that a latency period of 30 to 40 years can exist between carcinogen exposure and TCC development in humans, and differences in exposures between men and women many years ago can be reflected in current TCC occurrence. It is also not yet known why TCC is more common in dogs that have been altered in both sexes. Higher risk of other cancers has also been reported in dogs who have been spayed or neutered (Bryan et al. 2007; Cooley et al. 2002; Torres de la Riva et al. 2013; Villamil et al. 2009). These differences will likely be important in gaining a better understanding of the development process of TCC and other cancers, and dog studies could be very informative.

Examples of Opportunities for Dogs Studies of Translational Value

Studies of the Etiology and Prevention of TCC

Dogs offer an exceptional opportunity to study potential genetic and environmental risk factors for TCC and to develop and test early detection and early intervention strategies. This is important because the causes of TCC in humans are only partially understood. Approximately half of TCC cases in humans are thought to be due to exposure to cigarette smoke and chemicals in the workplace (Dietrich and Golka 2012; Droller 2006; Felknor and Delclos 2006). Exposure to herbicides, pesticides, and contaminants in agricultural chemical mixtures could also increase TCC risk, although not all studies support this role (Alvanja and Bonner 2012; Koutros et al. 2009; Singh et al. 2010). Genetic factors in humans have been associated with increased TCC risk, especially in relation to chemical exposures, although the overall understanding of the heritable genetic variants associated with TCC risk in humans is minimal (Burger et al. 2013; Franekova et al. 2008; Murta-Nascimento et al. 2007). More than half of people with TCC have no known environmental or genetic risk factors for the cancer (Burger et al. 2013). Studies are needed to further identify these factors and gene–environment interactions that increase TCC risk, and dog studies could prove crucially important in this regard.

The strong breed-associated risk in dogs can be exploited to elucidate heritable factors and gene–environment interactions that increase TCC risk. Previously reported breed risks include a 16-fold to 20-fold increased risk in Scottish terriers and a threefold to fivefold increased risk in West Highland white terriers, Shetland sheepdogs, beagles, and wire hair fox terriers when compared with the risk in mixed-breed dogs (Knapp and McMillan 2013). Although heritable factors are thought to play an important role in TCC risk in humans, with the tremendous genetic diversity in humans, groups of humans with this level of heritable risk for TCC have not been identified. Once heritable factors are identified in dogs, however, then those same factors can be investigated in humans (Parker et al. 2010).

To further characterize TCC risk in dogs, a search was recently conducted of the Veterinary Medical Data Base (VMDB), a database of case information from multiple

university veterinary teaching hospitals in the United States and Canada. The search was conducted of cases between1999 and 2010 using SNOMED, and the odds ratios (ORs) of TCC risk compared with the risk of mixed-breed dogs were determined. Analyses of the TCC cases confirmed the risk for TCC in previously reported breeds and also indicated risk in additional breeds (Table 3).

Among breeds that are uncommon or less common in the United States, there were additional breeds that also 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. Studies are ongoing to define the heritable factors that lead to higher TCC risk in dogs.

The VMDB analyses were also useful in the assessment of sex predilection for TCC. Previously, the ratio of female to male dogs with TCC has been reported to range from 1.71:1 to 1.95:1 (Knapp and McMillan 2013). Similarly, in the VMDB cases, the female/male ratio in mixed breed dogs was 1.9:1. In dogs in some of the high-risk breeds, however, the sex predilection did not exist or was less pronounced. The ratio of female/male dogs was 0.8:1 in Scottish terriers (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). In Dalmatians, the ratio of female/male dogs was 3.7:1, although this was based on only 19 cases, and further study in larger numbers of dogs would be indicated. The sex distribution significantly differed for Scottish terriers ($p = 0.003$, Fisher's exact test) compared with mixed-breed dogs in the VMDB study. The sex distributions for the other four breeds (Shetland sheepdogs, West Highland white terriers, beagles, and Dalmatians), however, did not significantly differ from that of mixed-breed dogs.

In addition to differences in sex across breeds, a significant difference ($p < 0.001$) in anatomic location of the TCC was noted between the high-risk breeds and mixed-breed dogs in the VMDB study. The localization of the TCC to the bladder alone, or to the bladder and urethra alone (vs. involvement of the prostate, ureter, and kidney), was less common in mixed-breed dogs than in the higher-risk breeds (Table 4). Further study is needed to determine the reasons for the differences in tumor location and the differences in sex predilection across breeds.

The VMDB study was also of interest in confirming the increasing hospital prevalence of TCC at university veterinary teaching hospitals. The proportion of the canine caseload that consists of TCC cases has continued to increase. This increasing proportionate morbidity was previously reported between 1975 and 1995 (Knapp, Glickman, Denicola, et al. 2000). In the more recent VMDB study, the prevalence of TCC cases continued to increase between 1999 and 2010, from 0.15% to 0.7% of all hospital patients (Figure 2). When Purdue University cases (where TCC cases are actively recruited) were excluded, the graph was not appreciably different. Although increasing awareness of the TCC problem and the fact that effective therapies exist could encourage referral to teaching hospitals, it is likely that a true biological increase in TCC risk also exists.

In addition to study of the genetic factors related to TCC risk, studies in dogs can define environmental risk factors for TCC. The more limited breadth of exposures in many dogs and the compressed lifespan in dogs compared with humans aid in the identification of environmental risk factors for the disease. In addition, because dogs do not smoke, they could be a more specific sentinel for other causes of TCC in humans, although the risk of secondhand smoke has not been fully defined in dogs. Examples of non-smoking-related risks for TCC are provided herein.

Table 3 Summary of analyses of Veterinary Medical Data Base records of dogs with transitional cell carcinoma (TCC) and dogs in the same breed without TCC (SNOMED search, 1999–2010)

Breed	Number of dogs in that breed in data base	TCC cases in that breed in data base	OR compared with 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

The odds ratios (ORs) of TCC risk compared with 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.

Table 4 Location of transitional cell carcinoma (TCC) within the urinary tract in mixed breed dogs and in dogs with high breed-associated risk for TCC

Location	Scottish terrier	Shetland sheepdog	West Highland white terrier	Beagle	Dalmatian	Mixed breed
Bladder only	76%	68%	77%	64%	63%	48%
Bladder and urethra	11%	17%	14%	15%	11%	19%
Urethra	1%	4%	5%	6%	26%	17%
Other[a]	11%	11%	5%	15%	0%	16%

[a]Could include prostate, kidney, or ureter.

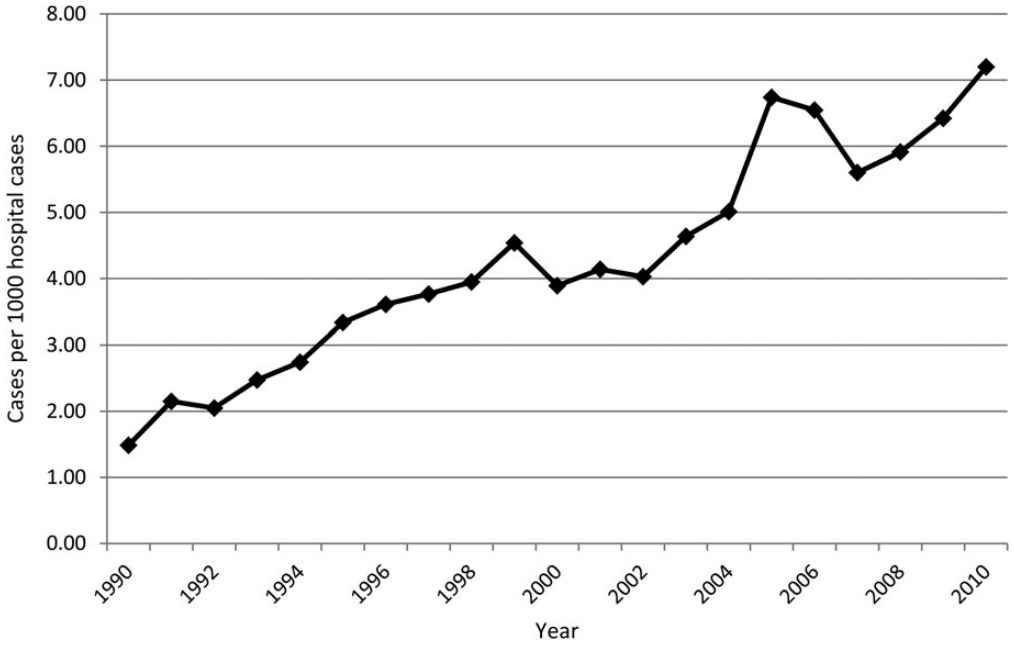

Figure 2 University hospital prevalence of canine bladder cancer expressed as rate per 1000 dogs evaluated at the teaching hospitals (Veterinary Medical Data Base).

In a previous case–control study, a significant association between TCC risk and exposure to the topical application of flea and tick dips was noted (Glickman et al. 1989). In the highest-risk group (overweight female dogs), the risk for TCC was 28 times that of normal-weight male dogs not exposed to the insecticides. The exact chemical carcinogen was not determined in this study. In fact, the authors speculated that the inert ingredients (solvents and petroleum distillates), which accounted for more than 95% of the product, could be at least partially responsible for the risk. Fortunately, newer, spot-on types of flea control products appear safer (Raghavan et al. 2004).

Another environmental risk for TCC in dogs is exposure to lawn chemicals, with a strong association found for lawn chemical exposure and TCC risk in the genetically susceptible dog breed of Scottish terriers (Glickman et al. 2004). In a case–control study, Scottish terriers exposed to lawn herbicides had a 3.6-fold increased risk for TCC (OR = 3.62; 95% confidence interval [CI] = 1.17–11.19; $p = 0.03$) compared with unexposed dogs. Dogs exposed to lawn herbicides and pesticides had a 7.2-fold increased TCC risk (OR = 7.19; 95% CI = 2.15–24.07; $p = 0.001$) (Glickman et al. 2004). It was suggested that chemical carcinogens (or precarcinogens) on the lawn were internalized by the dogs and excreted in urine, thereby exposing the urothelium to harmful chemicals.

A follow-up study was performed to prospectively measure herbicide concentrations in the urine of dogs living in households that did or did not apply lawn chemicals (Knapp, Peer, et al. 2013). Briefly, the concentrations of three chemicals commonly used in commercial lawn care products, including 2,4-dichlorophenoxyacetic acid, 4-chloro-2methylphenoxypropionic acid, and dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl) pyridine-3,5-dicarbothioate, were measured. Concentrations of the three chemicals were measured in the urine of dogs and on the surface of the grass before lawn application and at 24 and 48 hours after chemical

application. It was concerning that at least one of the three chemicals was detected in the urine of dogs in 19 of 25 households after lawn treatment. Of greater concern, chemicals were also detected in the urine of dogs in 14 of 25 households before the lawn was treated and in dogs in four of eight untreated households. Chemicals were commonly detected in grass residues from treated and untreated lawns, suggesting chemical drift from nearby treated areas. It was also noted that the condition of the lawn affected chemical residence time. Concern has been expressed for higher TCC risk in people exposed to herbicides, pesticides, and contaminants in agricultural chemical mixtures, although not all studies support this (Alavanja and Bonner 2012; Boers et al. 2010; Koutros et al. 2009; Singh et al. 2010; Yi 2013).

In work related to environmental risk factors, dogs can be used to identify previously unknown environmental risk and to serve as sentinels for environmental exposures that could also be of risk to humans. The latency period between chemical exposure and TCC development can be as short as a year in dogs (range = 1–10 years) (Okajima et al. 1981), whereas latency periods in humans can extend to several decades (Dietrich and Golka 2012; Droller 2006; Felknor and Delclos 2006). Dogs could possibly serve as sentinels for environmental exposures in humans that could lead to higher TCC risk if not addressed in a timely fashion. Dogs have already been identified as sentinels for other cancer risk, with a notable example being mesothelioma from exposure to asbestos (Glickman et al. 1983; Kelsey et al. 1998). In another example, dogs living near toxic Superfund sites had higher genotoxic changes (micronuclei in cultured peripheral blood lymphocytes) than dogs living in other areas (Backer et al. 2001).

Studies of dogs with breed-associated risk for TCC can be extended to study early detection and intervention strategies. When conducting prevention research, it is extremely helpful to work with a well-defined group of individuals in which many of the subjects are expected to develop cancer in the study period. The breed-associated risk for TCC offers this opportunity. In general terms, a cohort of dogs could be selected based on breed and age to screen with ultrasound, urine sediment, and other tests at set intervals. The suggestion of neoplastic changes could be followed by pathologic confirmation by cystoscopy. Dogs with pathologic changes such as dysplasia or carcinoma in situ could then become the subjects for an interventional study, with the goal to prevent the progression to invasive TCC. This would allow the assessment of early detection and intervention strategies that could then be tested in humans, especially as groups of humans with high risk are identified. A study that would include a 30-year window of exposure and intervention in humans could be done in less than 3 years in dogs. It is very appealing, therefore, to conduct the dog studies first to select the most promising approach to then test in humans. Early detection and intervention studies are in the planning stages in dogs at risk for TCC. Future dog work could also include studies to test primary prevention strategies, that being strategies to prevent even the earliest pathologic changes. Although these studies could require more than 3 years to accomplish, the time frame would still be considerably shorter than the decades that would be required for similar studies in humans.

Investigation of Cellular and Molecular Features: Androgen and Estrogen Receptors

In addition to the examples provided above, canine TCC continues to serve as a model for the study of cellular and molecular events involved in TCC development and progression.

An emerging area of interest in bladder cancer is the role of hormone receptors, especially androgen receptors (ARs) and estrogen receptors (ERs) (Chang et al. 2013; Li, Izumi, Miyamoto 2012; Rahmani et al. 2013). These receptors are located throughout the body, including the bladder. In addition to their physiologic roles in normal bladder function, androgens and estrogens and the complexes formed with their receptors serve as important transcription factors for several genes involved in cell proliferation and survival (Chang et al. 2013; Li, Izumi, Miyamoto 2012; Maximov et al. 2013; Rahmani et al. 2013). Although the roles of AR signaling in prostate cancer (MacVicar and Hussain 2013), and ER signaling in breast cancer (Maximov et al. 2013) and therapies that target these signaling pathways have been widely appreciated for decades, the recognition of their potential importance in other cancers has been relatively recent (Chang et al. 2013).

The evidence that ARs can enhance TCC development and progression comes from in vitro and in vivo studies (Chang et al. 2013; Li, Izumi, Miyamoto 2012; Rahmani et al. 2013). Androgens have enhanced the proliferation of AR-expressing TCC cell lines. In rodent studies in which bladder tumors were induced by the tobacco product N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN), male animals formed tumors more often and more rapidly than female animals (Miyamoto et al. 2007). Similarly, castrated mice had fewer tumors than intact mice, and AR knockout mice did not form tumors in response to BBN (Li, Izumi, Miyamoto 2012; Miyamoto et al. 2007). A proportion of human TCCs express ARs, although the exact percentage has not been defined. Immunohistochemistry studies have indicated AR expression in 13–78% of human TCC cases (Li, Izumi, Miyamoto 2012; Miyamoto, Yao, et al. 2012). Some of the variation in the findings is likely due to different tissue processing methods, which can affect results. In a recent study of 188 TCC cases in humans, AR expression was noted in 42% of tumors from men and 43% of tumors from women and in 55% of low-grade tumors and 36% of high-grade tumors (Miyamoto, Yao, et al. 2012). AR expression in the more "normal" tissues adjacent to the tumor (80%) was higher than in TCC. Other investigators have reported higher AR expression in organ-confined muscle invasive TCC than in metastatic disease (Gakis and Stenzl 2013). Thus, a trend appears to be emerging for higher AR expression in the normal bladder and less-aggressive forms of TCC and reduced expression in the higher-grade and -stage disease (Miyamoto, Yao, et al. 2012). Another important aspect of AR signaling is the finding of

AR variants that lack some part of the ligand binding domain but can signal in the absence of ligand binding (Li, Hwang, et al. 2012). Although AR variants have not been studied to any extent in TCC, AR variants in prostate cancer have been associated with hormone refractory disease, bone metastasis, and poor prognosis.

Dogs offer an intriguing opportunity to further study the role of AR signaling in TCC development and progression. In our laboratory, immunohistochemistry was performed as previously described (Dhawan, Ramos-Vara, Hahn, et al. 2013) using an anti-AR antibody (sc-816; Santa Cruz Biotechnology, Santa Cruz, CA). Two investigators (K.Y. and D.D.) reviewed all slides independently, and slides were considered positive when immunostaining was observed in 10% or more of tumor cells. AR expression was detected in 15 of 37 canine TCC specimens and in 10 of 15 normal bladder sections (Figure 3). AR expression in TCC was noted in 10 of 16 (62%) tissues from neutered male dogs, 0 of 1 tissue from an intact male dog, and 5 of 20 (25%) tissues from spayed female dogs. The downregulation of the AR gene in the high-grade cancer compared with the normal bladder in dogs was also confirmed in expression array studies (Dhawan, Ramos-Vara, Hahn, et al. 2013). Thus the trend of higher AR expression in the normal bladder and lower expression in more advanced human TCC appears to exist in dogs as well. It should be noted that the antibody used in the immunohistochemical studies and in Western blot analyses of canine samples is expected to bind to the full-length AR in dog samples (based on manufacturer report) but may not recognize AR variants. Using Western blot analysis, marked expression of AR (110 kd protein) was noted in 2 of 10 canine TCC cell lines (Dhawan, et al. 2009), with no to minimal expression in other lines (Figure 4). Using the same conditions, AR expression was noted in one of three human TCC cell lines (Figure 3). The addition of the androgen antagonist flutamide (0.1–25 μM; Sigma, St. Louis, MO) or the androgen agonist, R1881 (0.01–100 nM; Sigma) had no to little effect on the proliferation of three canine and two human TCC cell lines (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, assay of cells in androgen-depleted media), suggesting other signaling pathways were more dominant at that time. It is recognized that AR signaling may have different effects at different stages of TCC development. Further study of the entire process is indicated, and dogs could prove valuable in this research.

There is emerging evidence for the importance of estrogens and ER signaling in TCC (George et al. 2013; Hsu et al. 2013; Kauffman et al. 2013; Miyamoto, Yao, et al. 2012). ERα and ERβ are postulated to have different effects on TCC development. In rodent studies of BBN-induced bladder tumors, ERα appeared to have a protective role and ERβ an enhancing role in tumor development (Hsu et al. 2013), although in other studies ERα appeared important in tumor initiation (George et al. 2013). In humans, ERα expression has been reported in 50% of the normal bladder section adjacent to tumor, 38% of low-grade tumors, and 19% of high-grade, invasive tumors (Miyamoto, Yao, et al. 2012). Conversely, in human TCC, increasing ERβ expression is significantly associated with higher-grade and -stage tumors (Kauffman et al. 2013; Miyamoto, Yao, et al. 2012). ERβ has also been significantly associated with lymphovascular and perineural invasion, disease recurrence, and worse recurrence-free and overall survival after cystectomy (Kauffman et al. 2013). Activation of ERβ in bladder cancer cell lines has led to significant increases in proliferation, whereas treatment with tamoxifen was reported to inhibit cell growth (Kauffman et al. 2013).

The expression of ERs in canine TCC has also been noted. In work presented in abstract form, ERα expression was noted in approximately half of canine TCC cases and in all of canine normal bladders evaluated (McMillan, Dhawan, and Knapp, unpublished data). ERβ expression was noted in approximately three-quarters of canine TCC cases, as well as in the majority of normal bladder tissues from dogs. As discussed herein, most dogs with TCC in the United States have been spayed or neutered (as is common practice in veterinary medicine in this country), and most of the tissues studied were from spayed and neutered dogs. As research in AR and ER signaling continues, it will be important to

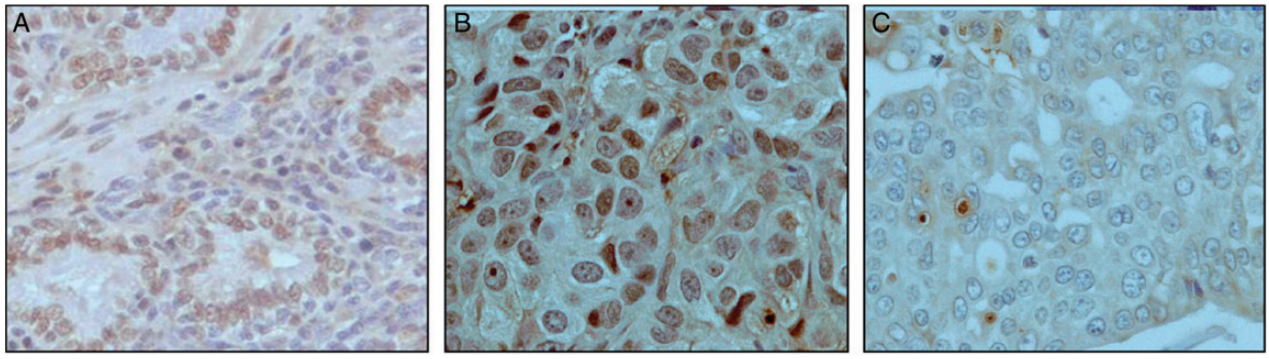

Figure 3 Expression of androgen receptors (ARs) in tissues. Immunohistochemistry was performed with anti-AR antibody (sc-816; Santa Cruz Biotechnology, Santa Cruz, CA) as described previously (Dhawan, Ramos-Vara, Hahn, et al. 2013). (A) Glandular cells in normal canine prostate served as a positive control. Note immunoreactivity observed as brown color detected by 3,3′-diaminobenzidine. AR expression was detected in the 40% of canine transitional cell carcinoma (TCC) sections (B), and was absent in 60% of TCC samples (C). Cells were counter stained with hematoxylin.

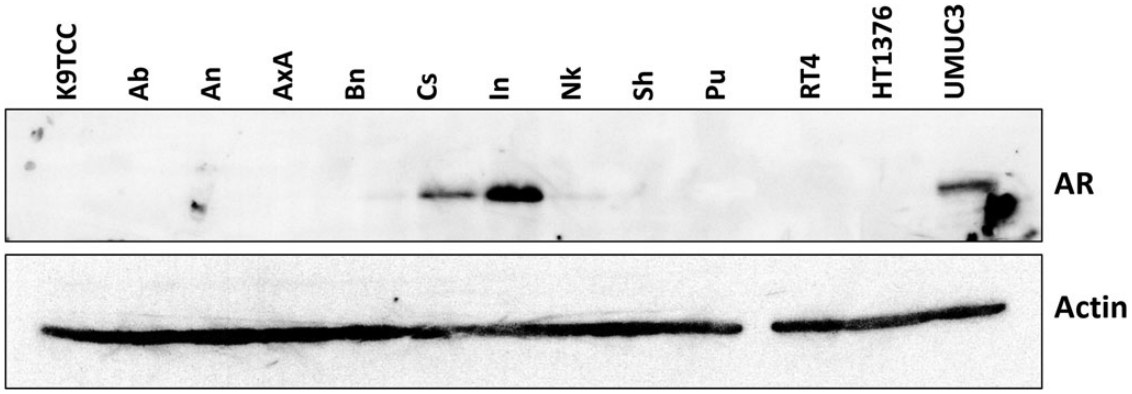

Figure 4 Western blot analyses of canine and human bladder cancer cell lines. Cell lysates (60 µg) were resolved on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and probed using anti–androgen receptor (AR) antibody (1:100, sc-816; Santa Cruz Biotechnology, Santa Cruz, CA). Two of 10 canine bladder cancer cell lines and one of three human bladder cancer cell lines expressed AR protein. Actin confirms equal protein loading.

include dogs from other countries in which more dogs remain sexually intact. It is likely that AR and ER signaling play an important role in TCC in humans and dogs, and studies involving dogs with TCC could prove highly valuable.

Treatment Trials in Dogs with TCC

One of the most important areas in which dog studies can provide translational value to ultimately help people with TCC is in treatment trials. Four examples of treatment studies in dogs with translational value are discussed: (1) cox inhibitor trials, (2) targeted therapy studies, (3) epigenetic based therapy, and (4) metronomic chemotherapy. The most progress has been made in the cox inhibitor studies, and these have received the most attention to date. An expanded summary of this area is included, along with more brief mention of the other therapies.

Cox Inhibitor Trials

Cox inhibitors are now widely accepted for their activity as cancer preventive and treatment agents in humans and dogs (Algra and Rothwell 2012; Knapp and McMillan 2013; Thun et al. 2012). Cox inhibitor studies in dogs are intriguing for several reasons. First, the antitumor effects of these drugs were observed and studied in dogs before major human studies (Knapp et al. 1992). Second, cox inhibitors have become a mainstay of TCC treatment in dogs (Knapp and McMillan 2013). Pet owners appreciate the oral treatment option, relatively low cost, and antitumor activity, along with the low risk of side effects. Thirds, cox inhibitor work has been translated from dogs to humans with similar biological effects seen in both species (Dhawan et al. 2010).

In canine TCC, it is well recognized that cox inhibitors have antitumor effects as single agents and enhance the activity of chemotherapy (Knapp and McMillan 2013). Single agent cox inhibitor studies have included the nonselective cox inhibitor piroxicam and the cox-2 selective inhibitors deracoxib, and firocoxib (Knapp and McMillan 2013; Knapp, Henry, et al. 2013; McMillan et al. 2011). All three agents induce remission (with most being partial remission) in approximately 15–20% of dogs with TCC. Disease stabilization can also be achieved in up to 55% of dogs.

The benefits of piroxicam treatment (0.3 mg/kg/day orally) in dogs with TCC has recently been confirmed in a larger number of dogs treated at the Purdue University Veterinary Teaching Hospital (PUVTH), including results from 34 dogs previously published (Knapp et al. 1994) and from 60 additional dogs evaluated and treated in a similar fashion at the PUVTH. A PUVTH medical records search between 1990 and 2013 was conducted to identify dogs with TCC that received single-agent piroxicam for all or part of their treatment regimen. All of the dogs had histopathologically diagnosed measurable TCC and had failed other therapies or were not eligible for other therapies. One difference between the original 34 cases and the newer cases is that in the newer cases, tumor size within the bladder was determined by a detailed ultrasound mapping protocol, as previously described (Hahn et al. 2012).

The 94 dogs treated with piroxicam ranged in age from 3.6 to 16.1 years (mean = 10.7 ± 2.5 years) and included 33 neutered male dogs (35%), 4 intact male dogs (4%), 56 spayed female dogs (60%), and 1 intact female dog (1%). The breeds included 22 mixed breed dogs, 13 Scottish terriers, 10 Shetland sheepdogs, 4 West Highland white terriers, 4 beagles, 3 Keeshonds, 3 German shepherds, 2 Cairn terriers, 2 Irish setters, 3 dachshunds, 2 cocker spaniels, 2 golden retrievers, and 1 each of Fox terrier, Pharoh hound, Basset hound, Jack Russell terrier, Pomeranian, coonhound, shar pei, Pembroke Welsh corgi, Dalmatian, miniature pinscher, American Eskimo, Labrador retriever, schnauzer, Basenji, Yorkshire terrier, French bulldog, boxer, collie, Australian shepherd, shih tzu, Wheaten terrier, miniature poodle, Pekingese, and Brittany spaniel. The TNM stages included 69 T2N0M0, 3 T2N1M0, 3 T2N0M1, 4 T2N1M1, 10 T3N0M0, 1 T3N1M0, 2 T3N0M1, 1 T3N1M1, and 1 T0N0M1 tumor. Metastases were detected in 16% of the dogs, including lymph node metastases in 9.5% of dogs and

distant metastases in 11.5% of dogs. The tumor involved the urethra in 40% of dogs and the prostate in 32% of male dogs. The tumor response was known in 76 dogs and included 2 (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). In the other 18 dogs, some follow-up information was available but not specific imaging results. The median progression-free interval (PFI) from the start of piroxicam until progressive disease occurred was 120 days (range = 17–1256 days) (Figure 5). The median survival from the start of piroxicam until death was 244 days (range = 6–1256 days) (Figure 6). Other treatments were given in 29 dogs after single-agent piroxicam, and these treatments included mitoxantrone (n = 6 dogs), cisplatin (n = 6 dogs), vinblastine (n = 5 dogs), chlorambucil (n = 5 dogs), deracoxib (n = 4 dogs), doxorubicin (n = 3 dogs), and zebularine (n = 2 dogs). One dog each received carboplatin, firocoxib, cyclophosphamide, actinomycin D, dacarbazine, folate-vinblastine, and mycobacterial cell wall DNA complex. The median survival did not differ significantly ($p = 0.24$) between dogs who received other therapies after piroxicam (344 days) and dogs who did not receive other therapies (176 days). None of the potential variables of interest (T stage, N stage, M stage, any metastasis, or at-risk breed) were significantly associated with PFI or survival (Cox proportional hazard analysis) or remission (chi-square analysis). Of 55 dogs in which information was available regarding the cause of death, death (natural death or euthanasia) was due to the primary tumor in 29 dogs (53%), metastatic disease in 11 dogs (20%), combined primary and metastatic disease in 3 dogs (5%), and noncancer-related causes in 12 dogs (22%).

Piroxicam was generally well tolerated. Using criteria established by the Veterinary Cooperative Oncology Group (2004), adverse gastrointestinal events were noted in 29 dogs, including 10 dogs with grade 1, 16 dogs with grade 2, 1 dog with grade 3, and 2 dogs with grade 4 adverse events. The alanine aminotransferase increased from normal to abnormal in 10 dogs. Alkaline phosphatase, which has been noted to be elevated in many dogs with TCC, was above the reference range in 50 of 87 dogs before piroxicam treatment and increased from normal to abnormal in six additional dogs. In most cases, it was not possible to determine whether the adverse events and changes in alanine aminotransferase and alkaline phosphatase were due to the piroxicam, the cancer and resulting organ dysfunction, or other causes. Many owners commented that their dog felt much better on piroxicam. To follow-up on this, the medical records were reviewed for evidence of change in quality of life with piroxicam treatment. Information regarding changes in urination (frequency, urgency, stranguria, hematuria in the absence of infection), appetite, and activity level was compiled. Information regarding change in quality of life with piroxicam treatment was found in the records of 56 dogs. Of the

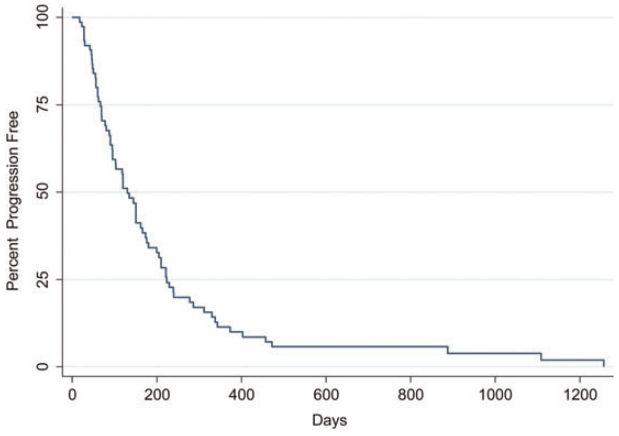

Figure 5 Kaplan–Meier curve for progression-free interval in dogs with transitional cell carcinoma treated with single-agent piroxicam. The median progression-free interval was 120 days (range = 17–1256 days).

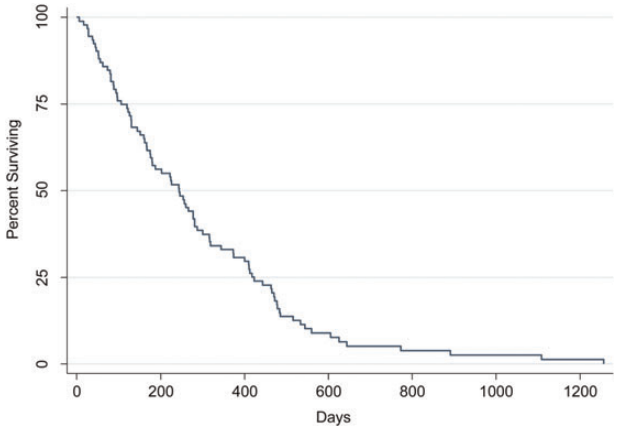

Figure 6 Kaplan–Meier curve for survival from the beginning of single-agent piroxicam treatment until death in dogs with transitional cell carcinoma. The median survival was 244 days (range = 6–1256 days).

56 dogs, the quality of life improved in at least one area (and did not worsen in any area) in 36 dogs, remained the same in 14 dogs, and worsened in 6 dogs.

With the positive findings of cox inhibitor treatment in dog studies, translation to humans was an obvious next step. Careful consideration was taken to design a study to evaluate cox inhibitor effects and not interfere with the standard-of-care therapy in humans. The front-line treatment for people with invasive TCC is cystectomy with or without perioperative chemotherapy (Lerner et al. 2006). Patients who develop metastatic disease are treated with chemotherapy. Options for cox inhibitor studies in people with TCC included (1) a short-term intervention study between diagnosis and cystectomy, (2) a randomized trial in which patients receive cox inhibitor or placebo after cystectomy, (3) a randomized trial in which patients with metastatic disease receive chemotherapy or

chemotherapy plus cox inhibitor, and (4) a trial of cox inhibitor treatment after other treatments have failed. To initiate studies in humans, a short-term intervention study of a cox inhibitor between diagnosis and cystectomy was performed (Dhawan et al. 2010). Briefly, the cox-2 inhibitor celecoxib was given to humans with invasive TCC for 2 to 6 weeks between diagnosis (by cystoscopic biopsy) and the scheduled cystectomy. Recognizing that measuring change in tumor size over this limited period of time would be difficult, biological endpoints were selected, with the main endpoint being induction of apoptosis in the tumor tissue between the pretreatment biopsy and post-treatment cystectomy samples. Although the exact mechanisms of the antitumor activity of cox inhibitors have not been not fully defined, induction of apoptosis in the tumor has been a consistent finding in animal and human studies (de Groot et al. 2007; Dhawan et al. 2008; Mohammed et al. 2002; Mohammed et al. 2006). A significant association between increase in apoptotic index and tumor regression with piroxicam treatment has been observed in dogs with TCC (Mohammed et al. 2002). In the celecoxib trial, no residual invasive cancer was identified in 3 of 13 patients at the time of cystectomy (after celecoxib). Of the remaining 10 patients, 7 had induction of apoptosis in the tumor, demonstrating a similar biological effect to that observed in dogs with TCC.

In addition to the effects of single-agent cox inhibitor treatment, these drugs also enhance the activity of chemotherapy. In dogs, cox inhibitors have enhanced the activity of cisplatin in multiple studies, including randomized trials (Knapp, Glickman, Widmer, et al. 2000; Knapp, Henry, et al. 2013; Mohammed et al. 2003). The remission rate with cisplatin alone was less than 20%, whereas the remission rate with cisplatin and cox inhibitor ranged 50–70% (Knapp and McMillan 2013; Knapp, Glickman, Widmer, et al. 2000; Knapp, Henry, et al. 2013). Clearly, further study of the effects of cox inhibitors in TCC treatment is indicated, and dogs offer an ideal animal model for this work. It remains to be determined whether nonselective cox inhibitors or selective cox-2 inhibitors will emerge as more optimal agents.

Targeted Therapy for Bladder Cancer

The targeted therapy of cancer is becoming essential to have the greatest antitumor effects with minimal risk of toxicity and to develop more individualized treatment. Two examples are provided of targeted therapy approaches being investigated in dogs in which results are expected to be of translational value in humans. The first involves the investigation of folate-targeted therapy, an emerging approach exploiting the high uptake of folate (vitamin B9) and folate drug conjugates into certain cancers compared with normal tissues (Low et al. 2008). Folate receptor (FR) expression in normal tissues is limited, whereas upregulation of FRs has been noted in several forms of human cancer (Low et al. 2008; Parker et al. 2005; Reddy et al. 2007; Zhao et al. 2008). Trials are ongoing of folate-targeted therapy in humans with ovarian cancer and lung cancer, but this therapy has not yet been directed to human bladder cancer. A dog study was conducted to determine the potential value of folate-targeted therapy in bladder cancer (Dhawan, Ramos-Vara, Naughton, et al. 2013). Immunohistochemical studies were performed on canine and human TCC tissues, with comparison with normal bladder, and FRs were detected (PU17, polyclonal antibody; Endocyte, West Lafayette, IN) in most primary TCC tissues and many nodal and lung metastases from dogs, and scintigraphy confirmed folate uptake in primary and metastatic lesions. Immunoreactivity to PU17 was similar in humans (78% of primary TCC; 80% of nodal metastases). Less immunoreactivity to a monoclonal antibody (mab343; 22% of cases) occurred. Although FR-α has been the most widely studied FR in cancer (Low et al. 2008), FR-β was noted in 21% of human TCC cases. A dose-escalation study of folate-targeted vinblastine (EC0905; Endocyte) was conducted in dogs with biopsy-confirmed, FR-positive TCC. As is the case for all clinical trials in dogs, the study was approved by the institutional animal care and use committee, and informed pet owner consent in writing was obtained for all participating dogs. The maximum tolerated dose of EC0905 in dogs (0.25 mg/kg intravenously weekly) was determined with neutropenia at higher doses. In 10 dogs, the tumor responses included partial remission (≥50% reduction in tumor volume) in five dogs, and stable disease (<50% change in tumor volume) in four dogs. The tumor response could not be determined in one dog. With these initial promising results, further study of folate-targeted therapy in dogs is ongoing. Demonstrating the benefit in dogs will provide justification to expand the human application of folate-targeted therapy to bladder cancer, as well as the other cancers currently under study.

Another example of targeted therapy development relates to a bladder cancer–specific peptide (PLZ4; amino acid sequence: cQDGRMGFc) that specifically binds to human TCC cell lines and cells obtained from patients (Lin et al. 2011). This same peptide has been found to bind to five canine TCC cell lines with high affinity and to then internalize (Zhang H et al. 2012). Further studies are in progress to use this peptide as a targeting ligand to deliver therapeutic agents.

Epigenetic-Based Therapies: Demethylating Agent Trials

Epigenetic events that can drive cancer development and progression in the absence of DNA mutational events are becoming widely recognized as targets for cancer therapy (Baylin and Jones 2011). One of the key events in this process is aberrant methylation in the promoter region of tumor suppressor genes, resulting in silencing of the genes. 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, Ramos-Vara, Hahn, et al. 2013) and to be associated with more aggressive disease in humans (Wu et al. 2011). When considering the development of clinical trials of demethylating agents in humans, key information from dogs could be of value, such as the level of effectiveness, safety,

and treatment schedule. A clinical trial of the demethylating agent 5-azacitidine (5-AzaC) in dogs with TCC was performed (Hahn et al. 2012). Doses were escalated in two different dose schedules (daily treatment for 5 days once per month or daily treatment for 5 days twice per month). Of 18 dogs evaluable for tumor response, partial remission, stable disease, and progressive disease were observed in four (22.2%), nine (50.0%), and four (22.2%) dogs, respectively. Consistent 5-AzaC levels (205–857 ng/ml) were detected in urine. The maximum tolerated dose in each treatment schedule was determined with neutropenia at higher doses. This study demonstrated promising activity of a demethylating agent in dogs with TCC. Further work in dogs is ongoing, including studies of an oral agent and work to define optimal dosing for demethylating activity, which may be substantially lower than maximum tolerated doses.

Metronomic Chemotherapy Trial

Metronomic chemotherapy is briefly mentioned for three reasons. First, it provides an excellent example of making better use of existing drugs or the "repurposing" of existing drugs to treat TCC. Second, it highlights the opportunity to test treatment strategies in dogs that are expected to be beneficial but are not yet proven. Third, it provides an example of how TCC can be treated as a chronic disease with the goal to control the cancer rather than to attempt, largely unsuccessfully, to eradicate it. Briefly, metronomic chemotherapy consists of the administration of low doses of chemotherapeutic drugs frequently and repetitively to prevent or delay progression of cancer (Lien et al. 2013; Mutsaers 2009). Metronomic chemotherapy is thought to have effects through multiple mechanisms, including inhibition of the formation of new blood vessels in tumors and modulation of immune system function (Lien et al. 2013; Nars and Kaneno 2013). Metronomic chemotherapy is well tolerated by rodents and humans and has shown promise for slowing the growth of tumors, including tumors for which other treatments have been unsuccessful (Mutsaers 2009). Although remission is not typically expected, metronomic chemotherapy is expected to help control cancer for a long period of time (i.e., cancer is treated as a chronic disease that is managed rather than cured). Metronomic chemotherapy is expected to be of benefit in TCC but has received little attention in this cancer type. To help determine the potential value of metronomic chemotherapy in bladder cancer treatment, a study of metronomic chlorambucil (4 mg/m^2 orally every 24 hours) was undertaken in dogs with TCC (Schrempp et al. 2013). This dosage was selected from an earlier study of chlorambucil treatment in dogs with several types of cancer and is lower than the more traditional dose of chlorambucil (6 mg/m^2, with some dosing recommendations as high as 8 mg/m^2 in dogs) (Leach et al. 2012). Of 31 dogs enrolled in the study, 29 had failed previous therapies, although not the extensive treatments commonly given to humans with TCC. The tumor responses included 1 (3%) dog with partial remission (≥50% reduction in tumor volume), 20 (67%) dogs with stable disease (<50% change in tumor volume), and 9 (30%) dogs with progressive disease (≥50% increase in tumor volume or development of additional tumors); 1 dog was lost to follow-up. The median PFI from the start of chlorambucil treatment to disease progression was 119 days (range = 7–728 days). The median survival time of dogs from the time of the start of chlorambucil to death was 221 days (range = 7–747 days). Toxicity was minimal. In dogs, this treatment provided excellent quality of life and extended survival for several months in most dogs, offering a good conservative treatment option for TCC.

Conclusions

Naturally occurring invasive TCC in dogs offers a highly relevant animal model for studies to improve the outlook for people who have bladder cancer and for people who are at risk for developing bladder cancer. The great similarities between invasive TCC in humans and dogs afford excellent opportunities to study the etiology and cancer biology of TCC, to develop prevention strategies, to explore early detection and intervention approaches, and to investigate more effective therapies for the cancer. Numerous studies have been conducted in dogs, with successful results improving the care for pet dogs and being translated into human studies. These successes have established the foundation in this area of research. There is tremendous potential to expand this comparative and translational research approach to improve the management of invasive bladder cancer in dogs and humans.

Acknowledgments

The authors thank the clinicians and staff of the Purdue Comparative Oncology Program for their excellent work with dogs with bladder cancer in the PUVTH and Patty Kirts for assistance with the manuscript preparation. The authors also thank Debbie Folks-Huber, Sue Hardy, and Kathy Ellis for assistance with the VMDB search and data. The authors gratefully acknowledge the owners of pet dogs with bladder cancer who have provided support for the work summarized in this article.

References

Abbo AH, Jones DR, Masters AR, Stewart JC, Fourez L, Knapp DW. 2010. Phase 1 clinical trial and pharmacokinetics of intravesical mitomycin C in dogs with localized transitional cell carcinoma of the urinary bladder. J Vet Intern Med 24:1124–1130.

Alavanja MC, Bonner MR. 2012. Occupational pesticide exposures and cancer risk: a review. J Toxicol Environ Health B Crit Rev 15:238–263.

Algra AM, Rothwell PM. 2012. Effects of regular aspirin on long-term cancer incidence and metastasis: A systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol 13:518–527.

Anderson CR, McNiel EA, Gillette EL, Powers BE, LaRue SM. 2002. Late complications of pelvic irradiation in 16 dogs. Vet Radiol Ultrasound 43:187–192.

Arantes-Rodrigues R, Colaço A, Pinto-Leite R, Oliveira PA. 2013. In vitro and in vivo experimental models as tools to investigate the efficacy of

antineoplastic drugs on urinary bladder cancer. Anticancer Res 33:1273–1296.
Arnold EA, Childress MO, Fourez LM, Tan KM, Stewart JC, Bonney PL, Knapp DW. 2011. Phase II clinical trial of vinblastine in dogs with transitional cell carcinoma of the urinary bladder. J Vet Intern Med 25:1385–1390.
Backer LC, Grindem CB, Corbett WT, Cullins L, Hunter JL. 2001. Pet dogs as sentinels for environmental contamination. Sci Total Environ 274:161–169.
Baylin SB, Jones PA. 2011. A decade of exploring the cancer epigenome—Biological and translational implications. Nat Rev Cancer 11:726–734.
Besaratinia A, Cockburn M, Tommasi S. 2013. Alterations of DNA methylome in human bladder cancer. Epigenetics 8:1013–1022.
Blackburn AL, Berent AC, Weisse CW, Brown DC. 2013. Evaluation of outcome following urethral stent placement for the treatment of obstructive carcinoma of the urethra in dogs: 42 cases (2004–2008). J Am Vet Med Assoc 242:59–68.
Blumenreich MS, Yagoda A, Natale RB, Watson RC. 1982. Phase II trial of vinblastine sulfate for metastatic urothelial tract tumors. Cancer 50:435–438.
Boers D, Portengen L, Bueno-de-Mesquita HB, Heederik D, Vermeulen R. 2010. Cause-specific mortality of Dutch chlorophenoxy herbicide manufacturing workers. Occup Environ Med 67:24–31.
Boria PA, Glickman NW, Schmidt BR, Widmer WR, Mutsaers AJ, Adams LG, Snyder PW, DiBernardi L, de Gortari AE, Bonney PL, Knapp DW. 2005. Carboplatin and piroxicam therapy in 31 dogs with transitional cell carcinoma of the urinary bladder. Vet Comp Oncol 2005 3:73–80.
Bryan JN, Keeler MR, Henry CJ, Bryan ME, Hahn AW, Caldwell CW. 2007. A population study of neutering status as a risk factor for canine prostate cancer. Prostate 67:1174–1181.
Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y. 2013. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 63:234–241.
Cancer Genome Atlas Research Network. 2014. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature doi:10.1038/nature12965.
Carvalho JC, Thomas DG, McHugh JB, Shah RB, Kunju LP. 2012. p63, CK7, PAX8 and INI-1: An optimal immunohistochemical panel to distinguish poorly differentiated urothelial cell carcinoma from high-grade tumors of the renal collecting system. Histopathology 60:597–608.
Cekanova M, Uddin MJ, Bartges JW, Callens A, Legendre AM, Rathore K, Wright L, Carter A, Marnett LJ. 2013. Molecular imaging of cyclooxygenase-2 in canine transitional cell carcinomas in vitro and in vivo. Cancer Prev Res (Phila) 6:466–476.
Chang C, Lee SO, Yeh S, Chang TM. 2013. Androgen receptor (AR) differential roles in hormone-related tumors including prostate, bladder, kidney, lung, breast, and liver. Oncogene Oncogene doi:10.1038/onc.2013.274.
Chen CH, Chen RJ. 2011. Prevalence of telomerase activity in human cancer. J Formos Med Assoc 110:275–289.
Cheng L, Davidson DD, Maclennan GT, Williamson SR, Zhang S, Koch MO, Montironi R, Lopez-Beltran A. 2010. The origins of urothelial carcinoma. Expert Rev Anticancer Ther 10:865–880.
Cheng L, MacLennan GT, Lopez-Beltrán A. 2012. Histologic grading of urothelial carcinoma: A reappraisal. Hum Pathol 43:2097–2108.
Childress MO, Adams LG, Ramos-Vara JA, Freeman LJ, He S, Constable PD, Knapp DW. 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). J Am Vet Med Assoc 239:350–356.
Cooley DM, Beranek BC, Schlittler DL, Glickman NW, Glickman LT, Waters DJ. 2002. Endogenous gonadal hormone exposure and bone sarcoma risk. Cancer Epidemiol Biomarkers Prev 11:1434–1440.
de Brito Galvao JF, Kisseberth WC, Murahari S, Sutayatram S, Chew DJ, Inpanbutr N. 2012. Effects of gemcitabine and gemcitabine in combination with carboplatin on five canine transitional cell carcinoma cell lines. Am J Vet Res 73:1262–1272.
de Groot DJ, de Vries EG, Groen HJ, de Jong S. 2007. Non-steroidal anti-inflammatory drugs to potentiate chemotherapy effects: From lab to clinic. Crit Rev Oncol Hematol 61:52–69.
Dhawan D, Craig BA, Cheng L, Snyder PW, Mohammed SI, Stewart JC, Zheng R, Loman RA, Foster RS, Knapp DW. 2010. Effects of short-term celecoxib treatment in patients with invasive transitional cell carcinoma of the urinary bladder. Mol Cancer Ther 9:1371–1377.
Dhawan D, Jeffreys AB, Zheng R, Stewart JC, Knapp DW. 2008. Cyclooxygenase-2 dependent and independent antitumor effects induced by celecoxib in urinary bladder cancer cells. Mol Cancer Ther 7:897–904.
Dhawan D, Ramos-Vara JA, Hahn NM, Waddell J, Olbricht GR, Zheng R, Stewart JC, Knapp DW. 2013. DNMT1: An emerging target in the treatment of invasive urinary bladder cancer. Urol Oncol 31:1761–1769.
Dhawan D, Ramos-Vara JA, Naughton JF, Cheng L, Low PS, Rothenbuhler R, Leamon CP, Parker N, Klein PJ, Vlahov IR, Reddy JA, Koch M, Murphy L, Fourez LM, Stewart JC, Knapp DW. 2013. Targeting folate receptors to treat invasive urinary bladder cancer. Cancer Res 73:875–884.
Dhawan D, Ramos-Vara JA, Stewart JC, Zheng R, Knapp DW. 2009. Canine invasive transitional cell carcinoma cell lines: In vitro tools to complement a relevant animal model of invasive urinary bladder cancer. Urol Oncol 27:284–292.
Dietrich HG, Golka K. 2012. Bladder tumors and aromatic amines—Historical milestones from Ludwig Rehn to Wilhelm Hueper. Front Biosci (Elite Ed) 4:279–288.
Dill AL, Eberlin LS, Costa AB, Zheng C, Ifa DR, Cheng L, Masterson TA, Koch MO, Vitek O, Cooks RG. 2011. Multivariate statistical identification of human bladder carcinomas using ambient ionization imaging mass spectrometry. Chemistry 17:2897–2902.
Dill AL, Ifa DR, Manicke NE, Costa AB, Ramos-Vara JA, Knapp DW, Cooks RG. 2009. Lipid profiles of canine invasive transitional cell carcinoma of the urinary bladder and adjacent normal tissue by desorption electrospray ionization imaging mass spectrometry. Anal Chem 81:8758–8764.
Droller MJ. 2006. Epidemiology of bladder cancer. In: Lerner SP, Schoenberg MP, Sternberg CN, eds. Textbook of Bladder Cancer. Oxon UK: Taylor and Francis. p 3–12.
Eissa S, Motawi T, Badr S, Zaghlool A, Maher A. 2013. Evaluation of urinary human telomerase reverse transcriptase mRNA and scatter factor protein as urine markers for diagnosis of bladder cancer. Clin Lab 59:317–323.
Epstein JI. 2010. Diagnosis and classification of flat, papillary, and invasive urothelial carcinoma: the WHO/ISUP consensus. Int J Surg Pathol 18:106S–111S.
Epstein JI, Amin MB, Reuter VR, Mostofi FK. 1998. The World Health Organization/ International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Am J Surg Pathol 22:1435–1448.
Felknor SA, Delclos GL. 2006. Occupational risk factors. In: Lerner SP, Schoenberg MP, Sternberg CN, eds. Textbook of Bladder Cancer. Oxon UK: Taylor and Francis. p 13–17.
Franekova M, Halasova E, Bukovska E, Luptak J, Dobrota D. 2008. Gene polymorphisms in bladder cancer. Urol Oncol 26:1–8.
Gakis G, Stenzl A. 2013. Gender-specific differences in muscle-invasive bladder cancer: The concept of sex steroid sensitivity. World J Urol 31:1059–1064.
Gamblin RM, Sagartz JE, Couto CG. 1997. Overexpression of p53 tumor suppressor protein in spontaneous arising neoplasms of dogs. Am J Vet Res 58:857–863.
George SK, Tovar-Sepulveda V, Shen SS, Jian W, Zhang Y, Hilsenbeck SG, Lerner SP, Smith CL. 2013. Chemoprevention of BBN-induced bladder carcinogenesis by the selective estrogen receptor modulator tamoxifen. Transl Oncol 6:244–255.
Glickman LT, Domanski LM, Maguire TG, Dubielzig RR, Churg A. 1983. Mesothelioma in pet dogs associated with exposure of their owners to asbestos. Environ Res 32:305–313.

Glickman LT, Raghavan M, Knapp DW, Bonney PL, Dawson MH. 2004. Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. J Am Vet Med Assoc 224:1290–1297.

Glickman LT, Schofer FS, McKee LJ, Reif JS, Goldschmidt MH. 1989. Epidemiologic study of insecticide exposures, obesity, and risk of bladder cancer in household dogs. J Toxicol Environ Health 28:407–414.

Haggag R, Farag K, Abu-Taleb F, Shamaa S, Zekri AR, Elbolkainy T, Khaled H. 2014. Low-dose versus standard-dose gemcitabine infusion and cisplatin for patients with advanced bladder cancer: A randomized phase II trial-an update. Med Oncol 31:811.

Hahn NM, Bonney PL, Dhawan D, Jones DR, Balch C, Guo Z, Hartman-Frey C, Fang F, Parker HG, Kwon EM, Ostrander EA, Nephew KP, Knapp DW. 2012. Subcutaneous 5-azacitidine treatment of naturally-occurring canine urothelial carcinoma: a novel epigenetic approach to human urothelial carcinoma drug development. J Urol 187:302–309.

Higgins JPT, Kaygusuz G, Wang L, Montgomery K, Mason V, Zhu SX, Marinelli RJ, Presti JC Jr, van de Rijn M, Brooks JD. 2007. Placental S100 (S100P) and GATA3: Markers for transitional epithelium and urothelial carcinoma discovered by complementary DNA microarray. Am J Surg Pathol 31:673–680.

Higuchi T, Burcham GN, Childress MO, Rohleder JJ, Bonney PL, Ramos-Vara JA, Knapp DW. 2013. Characterization and treatment of transitional cell carcinoma of the abdominal wall in dogs: 24 cases (1985–2010). J Am Vet Med Assoc. 242:499–506.

Hsu I, Chuang KL, Slavin S, Da J, Lim WX, Pang ST, O'Brien JH, Yeh S. 2013. Suppression of ERβ signaling via ERβ knockout or antagonist protects against bladder cancer development. Carcinogenesis 35:651–661.

Ismaili N, Amzerin M, Flechon A. 2011. Chemotherapy in advanced bladder cancer: current status and future. J Hematol Oncol 4:35.

Kauffman EC, Robinson BD, Downes M, Marcinkiewicz K, Vourganti S, Scherr DS, Gudas LJ, Mongan NP. 2013. Estrogen receptor-β expression and pharmacological targeting in bladder cancer. Oncol Rep 30:131–138.

Kelsey JL, Moore AS, Glickman LT. 1998. Epidemiologic studies of risk factors for cancer in pet dogs. Epidemiol Rev 20:204–217.

Kiupel M, Webster JD, Bailey KL, Best S, DeLay J, Detrisac CJ, Fitzgerald SD, Gamble D, Ginn PE, Goldschmidt MH, Hendrick MJ, Howerth EW, Janovitz EB, Langohr I, Lenz SD, Lipscomb TP, Miller MA, Misdorp W, Moroff S, Mullaney TP, Neyens I, O'Toole D, Ramos-Vara J, Scase TJ, Schulman FY, Sledge D, Smedley RC, Smith K, Snyder PW, Southorn E, Stedman NL, Steficek BA, Stromberg PC, Valli VE, Weisbrode SE, Yager J, Heller J, Miller R. 2011. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. Vet Pathol 48:147–155.

Knapp DW. 2006. Animal models: naturally occurring canine urinary bladder cancer. In: Lerner SP, Schoenberg MP, Sternberg CN, eds. Textbook of Bladder Cancer. Oxon UK: Taylor and Francis. p 171–175.

Knapp DW, Adams LG, Degrand AM, Niles JD, Ramos-Vara JA, Weil AB, O'Donnell MA, Lucroy MD, Frangioni JV. 2007. Sentinel lymph node mapping of invasive urinary bladder cancer in animal models using invisible light. Eur Urol 52:1700–1708.

Knapp DW, Glickman NW, Denicola DB, Bonney PL, Lin TL, Glickman LT. 2000. Naturally-occurring canine transitional cell carcinoma of the urinary bladder: A relevant model of human invasive bladder cancer. Urol Oncol 5:47–59.

Knapp DW, Glickman NW, Widmer WR, DeNicola DB, Adams LG, Kuczek T, Bonney PL, DeGortari AE, Han C, Glickman LT. 2000. Cisplatin versus cisplatin combined with piroxicam in a canine model of human invasive urinary bladder cancer. Cancer Chemother Pharmacol 46:221–226.

Knapp DW, Henry CJ, Widmer WR, Tan KM, Moore GE, Ramos-Vara JA, Lucroy MD, Greenberg CB, Greene SN, Abbo AH, Hanson PD, Alva R, Bonney PL. 2013. Randomized trial of cisplatin versus firocoxib versus cisplatin/firocoxib in dogs with transitional cell carcinoma of the urinary bladder. J Vet Intern Med 27:126–133.

Knapp DW, McMillan SK. 2013. Tumors of the urinary system. In: Withrow SJ, Vail DM, eds. Withrow and MacEwen's Small Animal Clinical Oncology, 5th ed. St. Louis: Elsevier–Saunders. p 572–582.

Knapp DW, Peer WA, Conteh A, Diggs AR, Cooper BR, Glickman NW, Bonney PL, Stewart JC, Glickman LT, Murphy AS. 2013. Detection of herbicides in the urine of pet dogs following home lawn chemical application. Sci Total Environ 456–457:34–41.

Knapp DW, Richardson RC, Bottoms GD, Teclaw R, Chan TC. 1992. Phase I trial of piroxicam in 62 dogs bearing naturally occurring tumors. Cancer Chemother Pharmacol 29:214–218.

Knapp DW, Richardson RC, Chan TCK, Bottoms GD, Widmer WR, DeNicola DB, Teclaw R, Bonney PL, Kuczek T. 1994. Piroxicam therapy in 34 dogs with transitional cell carcinoma of the urinary bladder. J Vet Intern Med 8:273–278.

Koutros S, Lynch CF, Ma X, Lee WJ, Hoppin JA, Christensen CH, Andreotti G, Freeman LB, Rusiecki JA, Hou L, Sandler DP, Alavanja MC. 2009. Heterocyclic aromatic amine pesticide use and human cancer risk: Results from the U.S. Agricultural Health Study. Int J Cancer 124:1206–1212.

Ku JH, Godoy G, Amiel GE, Lerner SP. 2012. Urine survivin as a diagnostic biomarker for bladder cancer: A systematic review. BJU Int 110: 630–636.

Langner C, Ratschek M, Tsybrovskyy O, Schips L, Ziguener R. 2003. P63 immunoreactivity distinguishes upper urinary tract transitional-cell carcinoma and renal-cell carcinoma even in poorly differentiated tumors. J Histochem Cytochem 51:1097–1099.

Leach TN, Childress MO, Greene SN, Mohamed AS, Moore GE, Schrempp DR, Lahrman SR, Knapp DW. 2012. Prospective trial of metronomic chlorambucil chemotherapy in dogs with naturally occurring cancer. Vet Comp Oncol 10:102–112.

Lei AQ, Cheng L, Pan CX. 2011. Current treatment of metastatic bladder cancer and future directions. Expert Rev Anticancer Ther 11:1851–1862.

Lerner SP, Schoenberg MP, Sternberg CN. 2006. Textbook of Bladder Cancer. Oxon UK: Taylor and Francis.

Li Y, Hwang TH, Oseth LA, Hauge A, Vessella RL, Schmechel SC, Hirsch B, Beckman KB, Silverstein KA, Dehm SM. 2012. AR intragenic deletions linked to androgen receptor splice variant expression and activity in models of prostate cancer progression. Oncogene 31:4759–4767.

Li Y, Izumi K, Miyamoto H. 2012. The role of the androgen receptor in the development and progression of bladder cancer. Jpn J Clin Oncol 42:569–577.

Lien K, Georgsdottir S, Sivanathan L, Chan K, Emmenegger U. 2013. Low-dose metronomic chemotherapy: A systematic literature analysis. Eur J Cancer 49:3387–3395.

Lin TY, Zhang H, Wang S, Xie L, Li B, Rodriguez CO Jr, de Vere White R, Pan CX. 2011. Targeting canine bladder transitional cell carcinoma with a human bladder cancer-specific ligand. Mol Cancer 10:9.

Low PS, Henne WA, Doorneweerd DD. 2008. Discovery and development of folic-acid-based receptor targeting for imaging and therapy of cancer and inflammatory diseases. Acc Chem Res 41:120–129.

MacVicar GR, Hussain MH. 2013. Emerging therapies in metastatic castration-sensitive and castration-resistant prostate cancer. Curr Opin Oncol 25:252–260.

Maximov PY, Lee TM, Jordan VC. 2013. The discovery and development of selective estrogen receptor modulators (SERMs) for clinical practice. Curr Clin Pharmacol 8:135–155.

McCleary-Wheeler AL, Williams LE, Hess PR, Suter SE. 2010. Evaluation of an in vitro telomeric repeat amplification protocol assay to detect telomerase activity in canine urine. Am J Vet Res 71:1468–1474.

McMillan SK, Boria P, Moore GE, Widmer WR, Bonney PL, Knapp DW. 2011. Antitumor effects of deracoxib treatment in 26 dogs with transitional cell carcinoma of the urinary bladder. J Am Vet Med Assoc 239:1084–1089.

Meuten DJ, Meuten TLK. 2014. Tumors of the urinary system. In: Meuten D, ed. Tumors in Domestic Animals, 5th ed. Hoboken NJ: Wiley.

Miller RS, Torti FM. 1992. Chemotherapy of advanced transitional-cell carcinoma of the bladder. Cancer Chemother Pharmacol 30:S99–S110.

Mitra AP, Datar RH, Cote RJ. 2006. Molecular pathways in invasive bladder cancer: New insights into mechanisms, progression, and target identification. J Clin Oncol 24:5552–5564.

Mitra AP, Hansel DE, Cote RJ. 2012. Prognostic value of cell-cycle regulation biomarkers in bladder cancer. Semin Oncol 39:524–533.

Miyamoto H, Izumi K, Yao JL, Li Y, Yang Q, McMahon LA, Gonzalez-Roibon N, Hicks DG, Tacha D, Netto GJ. 2012. GATA binding protein 3 is down-regulated in bladder cancer yet strong expression is an independent predictor of poor prognosis in invasive tumor. Hum Pathol 43:2033–2040.

Miyamoto H, Yang Z, Chen YT, Ishiguro H, Uemura H, Kubota Y, Nagashima Y, Chang YJ, Hu YC, Tsai MY, Yeh S, Messing EM, Chang C. 2007. Promotion of bladder cancer development and progression by androgen receptor signals. J Natl Cancer Inst 99: 558–568.

Miyamoto H, Yao JL, Chaux A, Zheng Y, Hsu I, Izumi K, Chang C, Messing EM, Netto GJ, Yeh S. 2012. Expression of androgen and oestrogen receptors and its prognostic significance in urothelial neoplasm of the urinary bladder. BJU Int 109:1716–1726.

Mohammed SI, Bennett PF, Craig BA, Glickman NW, Mutsaers AJ, Snyder PW, Widmer WR, DeGortari AE, Bonney PL, Knapp DW. 2002. Effects of the cyclooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. Cancer Res 62:356–358.

Mohammed SI, Craig BA, Mutsaers AJ, Glickman NW, Snyder PW, deGortari AE, Schlittler DL, Coffman KT, Bonney PL, Knapp DW. 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. Mol Cancer Ther 2:183–188.

Mohammed SI, Dhawan D, Abraham S, Snyder PW, Waters DJ, Craig BA, Lu M, Wu L, Zheng R, Stewart J, Knapp DW. 2006. Cyclooxygenase inhibitors in urinary bladder cancer: In vitro and in vivo effects. Mol Cancer Ther 5:329–336.

Mohammed SI, Knapp DW, Bostwick DG, Foster RS, Khan KN, Masferrer JL, Woerner BM, Snyder PW, Koki AT. 1999. Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. Cancer Res 59:5647–5650.

Mostofi K, Ito N, Weinstein R. 1986. Pathology: The need for standardization of pathological examination and reporting. In: Denis L, ed. Developments in Bladder Cancer. New York: Alan R. Liss. p 67–83.

Murta-Nascimento C, Schmitz-Dräger BJ, Zeegers MP, Steineck G, Kogevinas M, Real FX, Malats N. 2007. Epidemiology of urinary bladder cancer: from tumor development to patient's death. World J Urol 25:285–295.

Mutsaers AJ. 2009. Metronomic chemotherapy. Top Companion Anim Med 24:137–143.

Mutsaers AJ, Widmer WR, Knapp DW. 2003. Canine transitional cell carcinoma. J Vet Intern Med 17:136–144.

Nars MS, Kaneno R. 2013. Immunomodulatory effects of low dose chemotherapy and perspectives of its combination with immunotherapy. Int J Cancer 132:2471–2478.

Nolan MW, Kogan L, Griffin LR, Custis JT, Harmon JF, Biller BJ, Larue SM. 2012. Intensity-modulated and image-guided radiation therapy for treatment of genitourinary carcinomas in dogs. J Vet Intern Med 26:987–995.

Okajima E, Hiramatsu T, Hirao K, Ijuin M, Hirao Y, Babaya K, Ikuma S, Ohara S, Shiomi T, Hijioka T, Ohishi H. 1981. Urinary bladder tumors induced by N-butyl-N-(4-hydroxybutyl)nitrosamine in dogs. Cancer Res 41:1958–1966.

Orlando C, Gelmini S, Selli C, Pazzagli M. 2001. Telomerase in urological malignancy. J Urol 166:666–673.

Owen LN. 1980. TNM Classification of Tumours in Domestic Animals. Geneva: World Health Organization.

Parker HG, Shearin AL, Ostrander EA. 2010. Man's best friend becomes biology's best in show: Genome analyses in the domestic dog. Annu Rev Genet 44:309–336.

Parker N, Turk MJ, Westrick E, Lewis JD, Low PS, Leamon CP. 2005. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. Anal Biochem 338:284–293.

Patrick DJ, Fitzgerald SD, Sesterhenn A, Davis CJ, Kiupel M. 2006. Classification of canine urinary bladder urothelial tumours based on the World Health Organization/International Society of Urological Pathology consensus classification. J Comp Pathol 135:190–199.

Patronek GJ, Waters DJ, Glickman LT. 1997. Comparative longevity of pet dogs and humans: implications for gerontology research. J Gerontol A Biol Sci Med Sci 52:B171–B178.

Ploeg M, Aben KKH, Kiemeney LA. 2009. The present and future burden of urinary bladder cancer in the world. World J Urol 27:289–293.

Poirier VJ, Forrest LJ, Adams WM, Vail DM. 2004. Piroxicam, mitoxantrone, and coarse fraction radiotherapy for the treatment of transitional cell carcinoma of the bladder in 10 dogs: A pilot study. J Am Anim Hosp Assoc 40:131–136.

Raghavan M, Knapp DW, Bonney PL, Dawson MH, Glickman LT. 2005. Evaluation of the effect of dietary vegetable consumption on reducing risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. J Am Vet Med Assoc 227:94–100.

Raghavan M, Knapp DW, Dawson MH, Bonney PL, Glickman LT. 2004. Topical flea and tick pesticides and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. J Am Vet Med Assoc 225:38–394.

Rahmani AH, Alzohairy M, Babiker AY, Khan AA, Aly SM, Rizvi MA. 2013. Implication of androgen receptor in urinary bladder cancer: A critical mini review. Int J Mol Epidemiol Genet 4:150–155.

Ramos-Vara JA, Miller MA, Boucher M, Roudabush A, Johnson GC. 2003. Immunohistochemical detection of uroplakin III, cytokeratin 7, and cytokeratin 20 in canine urothelial tumors. Vet Pathol 40:55–62.

Rankin WV, Henry CJ, Turnquist SE, Turk JR, Beissenherz ME, Tyler JW, Rucker EB, Knapp DW, Rodriguez CO, Green JA. 2008. Identification of survivin, an inhibitor of apoptosis, in canine urinary bladder transitional cell carcinoma. Vet Comp Oncol. 6:141–150.

Reddy JA, Dorton R, Westrick E, Dawson A, Smith T, Xu LC, Vetzel M, Kleindl P, Vlahov IR, Leamon CP. 2007. Preclinical evaluation of EC145, a folate-vinca alkaloid conjugate. Cancer Res 67:4434–4442.

Reed LT, Knapp DW, Miller MA. 2013. Cutaneous metastasis of transitional cell carcinoma in 12 dogs. Vet Pathol 50:676–681.

Roberts JT, von der Maase H, Sengeløv L, Conte PF, Dogliotti L, Oliver T, Moore MJ, Zimmermann A, Arning M. 2006. Long-term survival results of a randomized trial comparing gemcitabine/cisplatin and methotrexate/vinblastine/doxorubicin/cisplatin in patients with locally advanced and metastatic bladder cancer. Ann Oncol 17:v118–v122.

Schrempp DR, Childress MO, Stewart JC, Leach TN, Tan KM, Abbo AH, de Gortari AE, Bonney PL, Knapp DW. 2013. Metronomic administration of chlorambucil for treatment of dogs with urinary bladder transitional cell carcinoma. J Am Vet Med Assoc 242:1534–1538.

Shariat SF, Karakiewicz PI, Godoy G, Karam JA, Ashfaq R, Fradet Y, Isbarn H, Montorsi F, Jeldres C, Bastian PJ, Nielsen ME, Müller SC, Sagalowsky AI, Lotan Y. 2009. Survivin as a prognostic marker for urothelial carcinoma of the bladder: A multicenter external validation study. Clin Cancer Res 15:7012–7019.

Shirahama T. 2000. Cyclooxygenase-2 expression is up-regulated in transitional cell carcinoma and its preneoplastic lesions in the human urinary bladder. Clin Cancer Res 6:2424–2430.

Singh BP, Nyska A, Kissling GE, Lieuallen W, Johansson SL, Malarkey DE, Hooth MJ. 2010. Urethral carcinoma and hyperplasia in male and female B6C3F1 mice treated with 3,3′,4,4′-tetrachloroazobenzene (TCAB). Toxicol Pathol 38:372–381.

Smee N, Loyd K, Grauer GF. 2013. UTIs in small animal patients: Part 2: Diagnosis, treatment, and complications. J Am Anim Hosp Assoc 49:83–94.

Swana HS, Grossman D, Anthony JN, Weiss RM, Altieri DC. 1999. Tumor content of the antiapoptosis molecule survivin and recurrence of bladder cancer. N Engl J Med 341:452–453.

Tanaka MF, Sonpavde G. 2011. Diagnosis and management of urothelial carcinoma of the bladder. Postgrad Med 123:43–55.

Thun MJ, Jacobs EJ, Patrono C. 2012. The role of aspirin in cancer prevention. Nat Rev Clin Oncol 9:259–267.

Torres de la Riva G, Hart BL, Farver TB, Oberbauer AM, Messam LL, Willits N, Hart LA. 2013. Neutering dogs: effects on joint disorders and cancers in golden retrievers. PLoS One 8:e55937.

Valli VE, Norris A, Jacobs RM, Laing E, Withrow S, Macy D, Tomlinson J, McCaw D, Ogilvie GK, Pidgeon G, Henderson RA. 1995. Pathology of canine bladder and urethral cancer and correlation with tumour progression and survival. J Comp Pathol 113:113–130.

Veterinary Co-operative Oncology Group. 2004. Veterinary co-operative oncology group—Common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.0. Vet Comp Oncol 2:194–213.

Vilar FO, de Araújo LA, Lima SV. 2004. Total bladder replacement with de-epithelialized ileum. Experimental study in dogs. Int Braz J Urol 30:237–244.

Villamil JA, Henry CJ, Hahn AW, Bryan JN, Tyler JW, Caldwell CW. 2009. Hormonal and sex impact on the epidemiology of canine lymphoma. J Cancer Epidemiol 2009:591753.

Wadhwa P, Goswami AK, Joshi K, Sharma SK. 2005. Cyclooxygenase-2 expression increases with the stage and grade in transitional cell carcinoma of the urinary bladder. Int Urol Nephrol 37:47–53.

Wallmeroth A, Wagner U, Moch H, Gasser TC, Sauter G, Mihatsch MJ. 1999. Patterns of metastasis in muscle-invasive bladder cancer (pT2-4): An autopsy study on 367 patients. Urol Int 62:69–75.

Willeit P, Willeit J, Mayr A, Weger S, Oberhollenzer F, Brandstätter A, Kronenberg F, Kiechl S. 2010. Telomere length and risk of incident cancer and cancer mortality. JAMA 304:69–75.

Wilson CR, Regnier FE, Knapp DW, Raskin RE, Andrews DA, Hooser SB. 2008. Glycoproteomic profiling of serum peptides in canine lymphoma and transitional cell carcinoma. Vet Comp Oncol 6:171–181.

Wu CT, Wu CF, Lu CH, Lin CC, Chen WC, Lin PY, Chen MF. 2011. Expression and function role of DNA methyltransferase 1 in human bladder cancer. Cancer 117:5221–5233.

Wu X, Kakehi Y, Taoka R, Tsunemori H, Inui M. 2005. Uroplakin II as a promising marker for molecular diagnosis of nodal metastases from bladder cancer: comparison with cytokeratin 20. J Urol 174:2138–2143.

Wu X, Kong XP, Pellicer A, Kreibich G, Sun TT. 2009. Uroplakins in urothelial biology, function, and disease. Kidney Int 75:1153–1165.

Wu X-R, Sun T-T, McConkey DJ, Shrader M, Papageorgiou A. 2006. Animal models of bladder cancer. In: Lerner SP, Schoenberg MP, Sternberg CN, eds. Textbook of Bladder Cancer. Oxon UK: Taylor and Francis. p 157–169.

Yagoda A. 1989. The role of cisplatin-based chemotherapy in advanced urothelial tract cancer. Semin Oncol 16:98–104.

Yi SW. 2013. Cancer incidence in Korean Vietnam veterans during 1992–2003: The Korean Veterans Health Study. J Prev Med Public Health 46:309–318.

Zhang H, Aina OH, Lam KS, de Vere White R, Evans C, Henderson P, Lara PN, Wang X, Bassuk JA, Pan CX. 2012. Identification of a bladder cancer-specific ligand using a combinatorial chemistry approach. Urol Oncol 30:635–645.

Zhang J, Wei S, Liu L, Nagana Gowda GA, Bonney P, Stewart J, Knapp DW, Raftery D. 2012. NMR-based metabolomics study of canine bladder cancer. Biochim Biophys Acta 1822:1807–1814.

Zhao X, Li H, Lee RJ. 2008. Targeted drug delivery via folate receptors. Expert Opin Drug Deliv 5:309–319.