Molecular Markers in Canine Urinary Bladder Cancer

Subjects: Veterinary Sciences | Urology & Nephrology
Contributor: Rui M. Gil da Costa

Cancer of the urinary bladder is a neoplasm with considerable importance in veterinary medicine, given its high incidence in several domestic animal species and its life-threatening character. Bladder cancer in companion animals shows a complex and still poorly understood biopathology, and this lack of knowledge has limited therapeutic progress over the years. The development and validation of Transitional cell carcinoma (TCC) molecular markers is of great importance for scientists and clinicians alike. Somatic and hereditary BRAF mutations received much attention and can now be detected via multiple types of tests, sometimes in useful combinations with CNA tests. Urine-based tests for detecting BRAF may allow the early detection of post-treatment relapse.

Keywords: transitional cell carcinoma ; urothelial carcinoma ; histology ; therapeutic marker ; prognosis ; biomarker ; BRAF ; dog

1. Prevalence and Aetiology

Urinary bladder cancer represents about 2 percent of all reported canine malign neoplasms [1][2], and it is rare in cats [3][4]. The majority of canine bladder tumours are malignant and of epithelial origin [5]. Transitional cell carcinoma (TCC), also referred to as urothelial carcinoma (UC) is the most frequent canine urinary bladder tumour [6]. The aetiology of the canine disease is thought to be multifactorial. Several risk factors have been proposed to play a role, such as exposure to older topical insecticides for flea and tick control, obesity, female sex, herbicides and breed predisposition (e.g., Scottish Terrier, West Highland White Terrier, Shetland Sheepdog, Beagle and others) [7][8][9].

Urinary bladder tumours are also frequently observed in cattle grazing on pastures infested by toxic ferns (mainly *Pteridium spp.*) (reviewed by Gil da Costa et al., 2012 [10]) and have been reported in other ruminant species [11]. The aetiology of bladder cancer in ruminants is much clearer than in companion animals. Grazing on poisonous ferns has been identified as a decisive risk factor since the mid-1900s [12][13]. The occurrence of bladder tumours in cattle is closely related to the geographical distribution of toxic ferns and originates in a syndrome known as bovine enzootic haematuria. Bladder lesions have also been reproduced experimentally in multiple laboratory animal models, by administering the fern or its toxin ptaquiloside [14][15][16][17][18]. Ptaquiloside is a DNA-alkylating agent, which causes point mutations, as well as structural and numeric chromosomal aberrations [19][20][21]. This toxin also has immunotoxic properties, contributing to reducing immune surveillance against newly arising neoplasms [22][23][24]. Other ferns containing ptaquiloside (e.g., *Pteris spp.* and *Dryopteris spp.*) or structurally related illudane toxins (for a review of illudane toxins, see Gil da Costa et al., 2013 [25]) do occur and have been reported to cause bladder cancer in cattle in various locations worldwide [26][27]. Bracken consumption has been proposed to facilitate a persistent abortive infection of the bovine bladder by bovine papillomavirus (BPV) types 1, 2, 13 and 14 [28][29][30][31]. These Delta BPV types are hypothesised to contribute to bladder carcinogenesis, by activating the platelet-derived growth factor receptor beta (PDGFR-β) through their oncoprotein E5 [32][33].

2. Histology and Grading

The urothelium is a hierarchically organised tissue, comprising basal, intermediate and umbrella cells, and the development of urothelial cancers progressively subverts this normal hierarchical structure [34]. Data obtained from human patients and from laboratory animals have helped trace different types of urothelial carcinoma to specific cell populations of origin and different differentiation pathways [35][36][37][38]. Although the pathogenesis of canine bladder cancer is less clear, it seems that numerous genetic changes involving key genes are shared between human and dogs, reflecting a conserved mechanism of pathogenesis [39]. Current laboratory models of bladder cancer, based on rats and mice, are out of the scope of the present work, but several comprehensive reviews have been recently published [40][41].

About 90 percent of all urinary bladder tumours in dogs are epithelial and malignant, and 50 to 90 percent of these will metastasise. Urinary bladder carcinomas include transitional cell carcinoma, squamous cell carcinoma, adenocarcinoma

and undifferentiated carcinoma (**Table 1**) [2]. Among primary epithelial neoplasms of the urinary bladder, TCC represents 75 to 90 percent in dogs [2]. More than 50 percent of overall malignant tumours show involvement of both the bladder and the urethra [5]. Benign epithelial tumours are rare, and only 10 percent of canine urinary bladder tumours are of mesenchymal origin, with smooth muscle neoplasms being the most common [2].

Table 1. Primary bladder tumours in dogs, types and percentages (the most common tumours reported are shown; adapted from Meuten and Meuten, 2016 [2]).

Primary Canine Urinary Bladder Tumours			
Epithelial			
Malignant	**(%)**	**Benign**	**(%)**
Transitional cell carcinoma	**75–90**	**Papilloma**	**2**
Undifferentiated carcinoma	**6**		
Adenocarcinoma	**4**	**Adenoma**	**0.2**
Squamous cell carcinoma	**3**		
Mesenchymal			
Malignant		**Benign**	
Leiomyosarcoma	**2**	**Leiomyoma**	**2**
Sarcoma	**1.5**		
Rhabdomyosarcoma	**1.3**		
Haemangiosarcoma	**1**	**Haemangioma**	**0.2**
Fibrosarcoma	**1**	**Fibroma**	**1**

Canine transitional cell carcinomas are classified based on their growth patterns (**Table 2**). They can be divided into papillary (papillary or cauliflower exophytic growths projected into the lumen) and non-papillary (plaques, flat nodules or masses), and into infiltrating or non-infiltrating tumours [2]. The consistent observation is that the majority (90 percent) of canine TCC shows an infiltrating growth pattern [2]. Papillary infiltrating TCC is one of the most common variants and likely to metastasise. The non-papillary and infiltrating type is the second or the most common variant in dogs, depending on the study, and shows a high tendency to metastasise [2].

Table 2. Classification of canine TCC subtypes based on growth pattern (adapted from Meuten and Meuten, 2016 [2]).

Canine Transitional Cell Carcinoma Classification
Papillary infiltrating
Often multiple and may cover large regions of the mucosa. Form papillary or exophytic growths that project into the lumen of the bladder. Invade the stalk and wall of the bladder, lamina propria, and muscle layers and may be transmural. Mild to marked cellular atypia. Likely to metastasise.
Papillary non-infiltrating
Do not invade the stroma of their own stalk, do not go beyond the lamina propria, so unlikely to metastasise. Differentiation from papilloma is subjective and based on criteria such as overall size, cellular atypia, small branches off the main lesion, among others. Non-invasive tumours may be adjacent to invasive TCC, and additional sections should be searched for invasion.
Non-papillary infiltrating
Form plaques and flat nodules, which can cover large regions of the mucosa. Surfaces are often ulcerated, tumour infiltrates into muscle layers, so high tendency to metastasise. Marked histological and cytological variability.
Non-papillary non-infiltrating
Rare. Additionally, defined as carcinoma in situ; confined to the epithelium and do not form papillae. Neoplastic epithelium more intensely eosinophilic than non-neoplastic cells; cells may be dysplastic to mildly anaplastic. Loss of intercellular cohesion. Usually located adjacent to invasive carcinoma; if seen, additional section analysis recommended to look for invasion.

In cattle, the histological features of bladder tumours are quite different, with 51.2% of purely epithelial tumours, 17.4% of purely mesenchymal tumours and 31.4% of coexisting epithelial and mesenchymal tumours, and numerous benign tumours (papillomas, haemangiomas, etc.) [42].

Over the years, several different grading systems for urothelial carcinomas in humans have been proposed and applied to veterinary tumours, looking for a better approach on the evaluation of the tumours' biologic behaviour [2][43]. Meuten and Meuten (2016) proposed a more simplified classification of TCC into low or high grade (Figure S1, Supplementary Material could be found in https://www.mdpi.com/2306-7381/9/3/107#supplementary). The majority of canine TCC are invasive, high grade and at an advanced stage when diagnosed [2]. In affected dogs, high-grade tumours seem to be more common in terriers than in non-terrier breeds [44].

3. Diagnosis

3.1. Clinical Signs and Differential Diagnosis

Clinical signs in dogs with TCC are usually nonspecific, many of which, such as dysuria, haematuria and pollakiuria, are commonly observed with urinary tract disease [2][5]. Concurrent urinary tract infections (UTI) are often present [2]. Tumour growth can lead to obstruction of the ureters or urethra and invasion and disruption of the normal functioning of the urethral sphincter [45]. On physical examination, a thickening of the urethra and of the trigone region of the bladder and enlargement of iliac lymph nodes may be found and, occasionally, a mass in the bladder or a distended bladder [1]. Urinary tract obstruction can occur prior to the development of lethal metastasis and is a common cause of death in dogs with TCC [6]. However, a normal physical examination does not exclude the presence of a TCC [46]. Differential diagnoses of canine TCC comprise other neoplasia, chronic cystitis, polypoid cystitis, fibroepithelial polyps, granulomatous cystitis/urethritis, calculi, among others [1].

In cattle, haematuria and weight loss are the main symptoms of bladder cancer and often present as part of the previously mentioned syndrome, known as bovine enzootic haematuria [26][47].

3.2. Diagnostic Procedures and Staging

Diagnostic procedures for TCC should include a complete blood cell count, serum biochemistry profile, urinalysis, urine culture (to rule out lower urinary tract infection) and cancer staging [1][46].

Definitive diagnosis of TCC can be established via histopathologic examination of tumour tissue and/or cytology of a representative sample [2]. Biopsies can be collected by means of cystotomy, cystoscopy and traumatic catheterisation, invasive procedures that usually involve general anaesthesia [1][39]. Cytological samples may be obtained by direct or ultrasound-guided percutaneous mass fine-needle aspirate or traumatic catheterisation [48].

The risk of tumour implantation or seeding/dissemination throughout other tissues following diagnostic or therapeutic procedures has been reported, especially after surgical manipulation of the tumour [49][50][51][52]. Even though reports are scarce, these should be carefully interpreted. Where possible, less invasive techniques should be favoured.

A less invasive technique consists of performing a cytology from urine sediment. If tumour cells are present, a diagnosis can be achieved [2]. However, negative results do not rule out TCC. In one study, malignant cells were seen in only 30% of dogs with lower urinary tract tumours [5]. Thus, cytological results must be interpreted with caution, especially upon the presence of inflammation of the urinary tract, and correlation with clinical data is essential for reaching a diagnosis.

Clinical staging of canine TCC includes thoracic and abdominal radiography, abdominal ultrasonography and specific urinary tract imaging [1][46]. Computer tomography (CT) has increasingly been used to aid in diagnostics and staging, particularly for more accurately evaluating the urethra and to detect metastases [53]. Figure S2 (Supplementary Material, could be found in https://www.mdpi.com/2306-7381/9/3/107#supplementary) shows the TNM (tumour, node, metastasis) classification for clinical staging of canine bladder cancer [54]. The TNM stage at diagnosis for TCC has shown to be strongly related to prognosis. More advanced TNM stage at the time of diagnosis was significantly associated with shorter survival [7][53]. Tumours located in the urethra were also associated with shorter survival time than ones in the bladder [53]. TCC has rarely been curable; however, with current therapies, many dogs will achieve stable disease for several months after diagnosis [1].

3.3. Recent Advances in Diagnostic Techniques for UC

As mentioned above, clinical presentation of canine TCC is comparable to several other (and far more common) urinary tract disorders. Consequently, the diagnosis of TCC is often delayed, allowing the tumour to grow, infiltrate and metastasise [2]. In fact, most TCCs are currently not diagnosed until they reach an advanced stage and thus present poor prognosis [55]. As such, effective (and preferably less invasive) methods for the early identification of UC are needed, which could improve responses to treatment and survival rates among affected dogs [56]. In particular, because TCC tumour cells and metabolites may be shed into urine, this body fluid is likely to present tumour-specific molecules that could be used as biomarkers for tumour detection using easily accessible samples collected through non-invasive techniques [46][57]. Over recent years, several potential biomarkers for canine TCC have been investigated for diagnostic/screening or prognostic purposes, and a few of them are currently available for commercial use.

These and other potential biomarkers are summarised in **Table 3** and detailed below.

Table 3. Current and potential markers for clinical applications in canine transitional cell carcinoma.

Biomarker	Sample	Method	Diagnostic Utility, Commercial Availability	Utility as a Prognostic and/or Therapeutic Target	Power of the Test	
					Sensitivity	Specificity
***BRAF* mutation**	**Tissue, urine, blood [58][59][60][61][62][63][64][65][66][67]**	**Determination of *cBRAF*^{V595E} mutation status in DNA retrieved from cells, using ddPCR analysis or other molecular methods.**	**Highly sensitive test for detecting TCC cells bearing the *BRAF* mutation. Could be used as a first, non-invasive screening test. Commercially available for dogs, for use in free-catch urine samples—CADET® *BRAF* mutation detection assay. Provides qualitative results (positive vs. negative for V595E) and quantitative data of tumour-derived mutation load in urine DNA. Reported to detect TCC in free-catch urine samples up to several months before development of clinical signs. The test is not affected by the presence of blood or bacteria in the urine. ~20% of tumours of canine TCC and PC patients do not possess the mutation, which limits the sensitivity of the ddPCR assay to ~80%. A more recent test that detects chromosomal copy number variation can be added in *BRAF* mutation-negative patients, increasing combined sensitivity to ~95% (CADET® *BRAF-PLUS).***	**BRAF mutation was not a predictor for histological grade, nor for survival. Measuring levels of BRAF mutation in urine or blood samples may be useful for monitoring treatment response and relapse. Potential target for treatment.**	**67–88% (TCC, tissue) 83–100% (TCC, urine)**	**100% (TCC, tissue and urine)**

Biomarker	Sample	Method	Diagnostic Utility, Commercial Availability	Utility as a Prognostic and/or Therapeutic Target	Power of the Test	
					Sensitivity	Specificity
BTA	Urine [68][69][70][71][72]	Rapid latex agglutination dipstick colorimetric test for qualitative detection of tumour analytes in urine. The test uses antibodies to detect a urinary bladder tumour-associated glycoprotein complex.	Useful as a screening test to rule out TCC, especially in dogs at high risk of developing TCC. False positive test results reported in dogs with non-neoplastic urinary tract disease, e.g., in the presence of significant glycosuria, proteinuria, and pyuria or haematuria. Presence of lower urinary tract malignant tumours other than TCC may yield positive results. Discrepancies with results may be observed over time, while reading the test. Commercially available —V-BTA Test. Results are either positive or negative. Not recommended as a confirmatory/definitive diagnostic test for urinary tract TCC in dogs, and should not be indiscriminately used in every patient presenting clinical signs of urinary tract disease.	N.A.	88–90%	35–41% in dogs with non-malignant urinary tract disease; 84–94% in healthy dogs or unhealthy dogs due to non-urinary tract diseases
bFGF	Urine [73][74][75][76][77][78]	ELISA urine test for human and canine bFGF. A quantitative sandwich enzyme immunoassay technique has also been developed using an antibody for canine bFGF.	Urine bFGF could be useful as a diagnostic tumour marker, helping to distinguish dogs with UTI from those with TCC. Commercially available (for research use, only): Quantikine® HS ELISA, Human FGF basic Immunoassay, Canine BFGF ELISA Kit, Nori® Canine FGF Basic ELISA Kit.	Quantification of urine bFGF could be useful as a non-invasive indicator of treatment response.	N.S.	N.S.

Biomarker	Sample	Method	Diagnostic Utility, Commercial Availability	Utility as a Prognostic and/or Therapeutic Target	Power of the Test	
					Sensitivity	Specificity
Chromosomal CNAs	Tissue, urine [39][79]	Assessment of urothelial cell ploidy/DNA copy number status in biopsy sections and in urine sediment by FISH.	Non-invasive method for canine TCC diagnosis. Potentially high-sensitivity and high-specificity FISH-based method/assay for the detection of canine TCC diagnosis utilising low-volume, free-catch urine specimens. Expensive and high effort method/labour intensive, expertise, time-consuming, increased cost, which may limit its application as routine diagnostics in a clinical environment. Not commercially available for canine TCC. Available for in vitro diagnostic use in human samples. A multicolour FISH-based assay for detection of aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus through FISH in urine specimens—UroVysion Bladder Cancer Kit.	N.A.	N.S.	N.S.
	Tissue, urine [55][64]	Multiplexed ddPCR assay for the detection and quantification of DNA copy number imbalances/changes characteristic to canine TCC.	Accurate, high-throughput method for evaluation of copy number changes in dogs with TCC. In this study, changes in copy number were not detected in 33% of urine DNA samples from dogs with TCC, which was probably due to the presence of inflammatory cells. Thus, additional techniques to improve sensitivity in those samples may be required. In such cases, FISH will still provide a more accurate evaluation. Commercially available for dogs, for use in free-catch urine samples: CADET® *BRAF-PLUS*. Can be used in *BRAF* mutation-negative patients. Could be added to CADET® *BRAF*, increasing combined sensitivity to ~95%.	N.A.	N.S.	N.S.

Biomarker	Sample	Method	Diagnostic Utility, Commercial Availability	Utility as a Prognostic and/or Therapeutic Target	Power of the Test	
					Sensitivity	Specificity
Microsatellite instability	Urine [72]	PCR study of a panel of 22 microsatellite DNA sequences from exfoliated urothelial cells and blood cells; comparison of microsatellites genotypes.	The technique added little value as a diagnostic test for TCC in dogs. High rate of false positives (32%, 12 of 38).	N.A.	55% (48% *)	68% (76%, vs. V-BTA) * When compared with results of V-BTA from the same study.
MicroRNAs	Tissue, cell lines [56]	QPCR of specific miRNAs involved in the pathophysiology of TCC in humans.	MiR-34a, miR-16, miR-103b and miR-106b could be useful diagnostic biomarkers for the identification of dogs with TCC. More studies are required, with a larger sample.	N.A.	N.S.	N.S.
	Blood, urine [80]		MiR-103b and miR-16 are potential non-invasive diagnostic biomarkers for TCC; particularly for distinguishing LUTD and TCC in canine urine samples. Urine tests seem to be superior in distinguishing TCC from LUTD.	N.A.	N.A.	N.S.
Telomerase	Canine TCC cell line, urine [81][82]	PCR-based telomeric repeat amplification protocol for detection/measurement of telomerase activity.	Telomerase activity may be useful in diagnosing canine TCC in urine samples in a clinical context. Results of the assay are either telomerase-positive or telomerase-negative. Urine samples containing other telomerase-positive cells may yield false-positive results (e.g., presence of activated lymphocytes in dogs with bacterial cystitis). False-negative results may occur with unappropriated urine samples storage.	N.A.	91% Diagnostic sensitivity/specificity of the TRAP assay applied to clinical canine urine samples.	89%

Biomarker	Sample	Method	Diagnostic Utility, Commercial Availability	Utility as a Prognostic and/or Therapeutic Target	Power of the Test	
					Sensitivity	Specificity
Calgranulins	Urine [44][83]	Species-specific radioimmunoassays to measure urine concentrations of canine calgranulins S100A8/A9 and S100A12.	Results presented as normalised to urine specific gravity levels ($S100A8/A9_{USG}$) and as S100A8/A9-to-S100A12 ratio (UcalR). Provides quantitative results. $S100A8/A9_{USG}$ could be a good a screening test for TCC/PC in dogs, especially in those where a UTI has been ruled out as a cause of clinical signs of lower urinary tract disease (due to a moderate rate of false positives observed for dogs ≥6 years of age with UTI). UcalR can help differentiate patients with a UTI from those with TCC/PC, even though a moderate false negative rate was seen in dogs ≥ 6 y.o. with a UTI. A combination of $S100A8/A9_{USG}$ and uCalR improved diagnostic accuracy for the detection of canine TCC/PC. Test levels are not affected by haematuria.	N.A.	96% $S100A8/A9_{USG}$ * 91% UcalR ** * For detection of TCC/PC in dogs ≥ 6 y.o.; ** to distinguish dogs with TCC/PC from dogs with UTI in dogs ≥ 6 y.o.	66% $S100A8/A9_{USG}$ * 60% UcalR **
Proteomics	Urine [84]	Characterisation of the canine urinary proteome by using liquid chromatography tandem mass spectrometry and immunoblot.	A protein signature was identified, that could distinguish between healthy patients and those with TCC or UTIs. A statistical model using a biomarker multiplex for categorising samples as TCC or non-TCC was developed, predicting the presence of disease with 90% confidence. Potential relevance of the identified proteins as biomarkers for the diagnosis of TCC in dogs. Preliminary study, high-throughput technique. A more direct assay will be useful for clinical diagnosis.	N.A.	N.S.	N.S.

Biomarker	Sample	Method	Diagnostic Utility, Commercial Availability	Utility as a Prognostic and/or Therapeutic Target	Power of the Test	
					Sensitivity	Specificity
Metabolomics	Urine [55]	Nuclear magnetic resonance spectroscopy-based metabolite profiling analysis.	Six metabolites showed significantly higher levels in dogs with TCC compared to controls: urea, choline, methylguanidine, citrate, acetone and β-hydroxybutyrate. Good sensitivity to predict the healthy control and disease samples. Potential for early detection of bladder cancer. Preliminary study, high-throughput technique.	N.A.	86%	78%
Lipidomics	Tissue [85]	Imaging analysis to examine lipidome/lipid profiles, using desorption electrospray ionisation mass spectrometry.	Differentiation of canine cancerous bladder tissue and cutaneous metastasis from noncancerous canine bladder tissue samples. Different lipid distributions between healthy and diseased tissues. DESI-MS imaging could be useful in diagnosing TCC by using a multimarker approach based on the lipid profiles and intensities of tissue samples. Further studies are required with larger populations and additional control groups, i.e., with other lower urinary diseases. Still requires invasive techniques for tissue collection.	N.A.	N.S.	N.S.
	Urine [86]	Analysis of lipid profiles using liquid chromatography-mass spectrometry.	Unique lipid profiles were found among dogs with TCC, dogs with UTI, and healthy dogs. Specific statistical analyses allowed their differentiation. Concentrations of the specific lipids could not be determined, and thus the study did not conclude which lipid families were up or downregulated. Foundation for further research on urinary lipids as potential biomarkers for TCC. Non-invasive method.	N.A.	N.S.	N.S.

Biomarker	Sample	Method	Diagnostic Utility, Commercial Availability	Utility as a Prognostic and/or Therapeutic Target	Power of the Test	
					Sensitivity	Specificity
Survivin	Tissue [87]	Immunohistochemistry for detection of survivin, an apoptosis-inhibiting protein; RT-PCR analysis for the survivin gene.	Initial phases of investigational development with limited samples. Additional research needed to investigate potential role of nuclear survivin as an early marker for bladder tumours, as well as in the development, progression and as a therapeutic target.	N.A.	N.S.	N.S.
EGFR	Tissue [88]	IHC and qPCR analysis for EGFR.	EGFR expression could potentially be used as a marker to aid canine TCC diagnosis. It may improve the sensitivity of urine cytological diagnosis when provisional diagnosis is needed.	Not useful for predicting prognosis of TCC.	72%	100%
HER-2	Tissue [61][89]	IHC for HER-2.	N.A.	Potential maker of malignancy and therapeutic target in canine TCC.	N.S.	N.S.
VEGFR2, PDGFR-β, c-KIT	Tissue, cell lines [90][91] [92][93] [94]	IHC for expression of VEGFR2, PDGFR-β, c-KIT.	PDGFR-β could play a role in canine TCC tumourigenesis.	PDGFR-β and VEGFR2 might be involved in mediating clinical response of TCC to toceranib.	N.S.	N.S.
Granzyme B, CD3	Tissue [95]	IHC and PCR assay for CD3 and granzyme B.	N.A.	Granzyme B^+ tumour-infiltrating cells could be involved in inhibition of tumour progression, and a favourable prognosis. Presence of granzyme B^+ tumour-infiltrating cells might be an independent prognostic factor.	N.S.	N.S.
P63, Ki67, β-catenin	Tissue [96][97] [98][99] [100][101] [102]	IHC for p63.	P63 could potentially be used as a clinical marker for diagnosing canine TCC.	P63 could potentially be used as a clinical marker for predicting prognosis in canine TCC.	N.S.	N.S.

Biomarker	Sample	Method	Diagnostic Utility, Commercial Availability	Utility as a Prognostic and/or Therapeutic Target	Power of the Test	
					Sensitivity	Specificity
UP III CK 7 CK 20 COX-2	**Tissue [2][44][103][104][105]**	**IHC for UP III, CK 7 and CK20.**	**UP III is the most common marker of urothelial differentiation used in dogs. It was considered the marker of choice in canine urothelial neoplasms. Although UP III is not a specific marker for TCC itself (it does not differentiate neoplastic from non-neoplastic lesions), it can be useful e.g., to rule in TCC in a biopsy from a tumour of unknown origin and to identify metastatic carcinomas in the skin. CK 7 was more sensitive than UP III for canine TCC, but CK 7 is expressed in several non-urothelial tumours and also in normal tissues, as is CK 20. CK 7 should be used for tumours negative for UP III but suspected of being TCC. CK 20 alone did not prove to be useful for diagnosis of urothelial tumours. Some urothelial carcinomas might not be positively labelled when using UP III and CK 7 as diagnostic markers. COX-2 has been found to be expressed in canine TCCs but not by normal urothelium of the urinary bladder.**	**UP III, CK 7, COX-2: Significant associations between specific patterns of expression and tumour classification, depth of neoplastic cell infiltration. COX-2: Intensity of COX-2 expression did not correlate with grading. Nonselective COX and COX-2 specific inhibitors have been used for treating TCC. Still unclear whether it could be useful as a predictive factor for treatment response.**	**N.S.**	**N.S.**

TCC: transitional cell carcinoma; PC: prostatic carcinoma; N.A.: Not available; N.S.: Not specified; y.o.: years old; vs.: versus.

References

1. Knapp, D.W.; McMillan, S.K. Tumors of the urinary system. In Withrow and MacEwen's Small Animal Clinical Oncology, 5th ed.; Withrow, S.J., Vail, D.M., Eds.; Elsevier–Saunders: St. Louis, MO, USA, 2013; pp. 572–582.
2. Meuten, D.J.; Meuten, T.L. Tumors of the urinary system. In Tumors in Domestic Animals, 5th ed.; Meuten, D.J., Ed.; John Willey & Sons Inc.: Ames, IA, USA, 2016; pp. 632–688.
3. Schwarz, P.D.; Greene, R.W.; Patnaik, A.K. Urinary bladder tumors in the cat: A review of 27 cases. J. Am. Anim. Hosp. Assoc. 1985, 21, 237–245.
4. Wilson, H.M.; Chun, R.; Larson, V.S.; Kurzman, I.D.; Vail, D.M. Clinical signs, treatments, and outcome in cats with transitional cell carcinoma of the urinary bladder: 20 cases (1990–2004). J. Am. Vet. Med. Assoc. 2007, 231, 101–106.
5. Norris, A.M.; Laing, E.J.; Valli, V.E.O.; Withrow, S.J.; Macy, D.W.; Ogilvie, G.K.; Tomlinson, J.; McCaw, D.; Pidgeon, G.; Jacobs, R.M. Canine Bladder and Urethral Tumors: A Retrospective Study of 115 Cases (1980–1985). J. Vet. Intern. Med. 1992, 6, 145–153.

6. Mutsaers, A.J.; Widmer, W.R.; Knapp, D.W. Canine Transitional Cell Carcinoma. J. Vet. Intern. Med. 2003, 17, 136–144.
7. Knapp, D.W.; Glickman, N.W.; DeNicola, D.B.; Bonney, P.L.; Lin, T.L.; Glickman, L.T. Naturally-occurring canine transitional cell carcinoma of the urinary bladder A relevant model of human invasive bladder cancer. Urol. Oncol. Semin. Orig. Investig. 2000, 5, 47–59.
8. Glickman, L.T.; Schofer, F.S.; McKee, L.J.; Reif, J.S.; Goldschmidt, M. Epidemiologic study of insecticide exposures, obesity, and risk of bladder cancer in household dogs. J. Toxicol. Environ. Health Part A 1989, 28, 407–414.
9. Glickman, L.T.; Raghavan, M.; Knapp, D.W.; Bonney, P.L.; Dawson, M.H. Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. J. Am. Vet. Med. Assoc. 2004, 224, 1290–1297.
10. Gil da Costa, R.; Bastos, M.; Oliveira, P.; Lopes, C. Bracken-associated human and animal health hazards: Chemical, biological and pathological evidence. J. Hazard. Mater. 2012, 203–204, 1–12.
11. Somvanshi, R. Papillomatosis in Buffaloes: A Less-Known Disease. Transbound. Emerg. Dis. 2011, 58, 327–332.
12. Rosenberger, G.; Heeschen, W. Adler-farn (Pteris aquiline)—Die ursache des sog. Dtsch. Tierarztl. Wochenschr. 1960, 67, 201–208.
13. Rosenberger, G. Längere aufnahme von adlerfarn (Pteris aquilina)—Die ursache der chronischen vesikalen haematurie des rindes. Wien. Tierarztl. Monatsschr. 1965, 52, 415–421.
14. Evans, I.A.; Mason, J. Carcinogenic Activity of Bracken. Nature 1965, 208, 913–914.
15. Pamukcu, A.M.; Price, J.M. Induction of Intestinal and Urinary Bladder Cancer in Rats by Feeding Bracken Fern (Pteris aquilina). JNCI J. Natl. Cancer Inst. 1969, 43, 275–281.
16. Hirono, I.; Ogino, H.; Fujimoto, M.; Yamada, K.; Yoshida, Y.; Ikagawa, M.; Okumura, M. Induction of Tumors in ACI Rats Given a Diet Containing Ptaquiloside, a Bracken Carcinogen2. JNCI J. Natl. Cancer Inst. 1987, 79, 1143–1149.
17. da Gil Costa, R.M.; Oliveira, P.A.; Vilanova, M.; Bastos, M.M.; Lopes, C.C.; Lopes, C. Ptaquiloside-induced, B-cell lymphoproliferative and early-stage urothelial lesions in mice. Toxicon 2011, 58, 543–549.
18. da Gil Costa, R.M.; Oliveira, P.A.; Bastos, M.M.S.M.; Lopes, C.C.; Lopes, C. Ptaquiloside-induced early-stage urothelial lesions show increased cell proliferation and intact β-catenin and E-cadherin expression. Environ. Toxicol. 2012, 29, 763–769.
19. Ojika, M.; Wakamatsu, K.; Niwa, H.; Yamada, K. Ptaquiloside, a potent carcinogen isolated from bracken fern var: Structure elucidation based on chemical and spectral evidence, and reactions with amino acids, nucleosides, and nucleotides. Tetrahedron 1987, 43, 5261–5274.
20. Pereira, L.O.; Bicalho, L.S.; Lopes, M.C.-D.; De Sousa, T.M.M.; Báo, S.N.; Santos, M.D.F.M.A.; Fonseca, M.J.P. DNA damage and apoptosis induced by Pteridium aquilinum aqueous extract in the oral cell lines HSG and OSCC-3. J. Oral Pathol. Med. 2008, 38, 441–447.
21. da Gil Costa, R.M.; Coelho, P.; Sousa, R.; Bastos, M.M.; Porto, B.; Teixeira, J.P.; Malheiro, I.; Lopes, C. Multiple genotoxic activities of ptaquiloside in human lymphocytes: Aneugenesis, clastogenesis and induction of sister chromatid exchange. Mutat. Res. Toxicol. Environ. Mutagen. 2012, 747, 77–81.
22. Latorre, A.O.; Furlan, M.S.; Sakai, M.; Fukumasu, H.; Hueza, I.M.; Haraguchi, M.; Górniak, S.L. Immunomodulatory effects of Pteridium aquilinumon natural killer cell activity and select aspects of the cellular immune response of mice. J. Immunotoxicol. 2009, 6, 104–114.
23. Caniceiro, B.D.; Latorre, A.O.; Fukumasu, H.; Sanches, D.S.; Haraguchi, M.; Górniak, S. Immunosuppressive effects of Pteridium aquilinumenhance susceptibility to urethane-induced lung carcinogenesis. J. Immunotoxicol. 2014, 12, 74–80.
24. Santos, C.; Ferreirinha, P.; Sousa, H.; Ribeiro, J.; Bastos, M.M.; Neto, T.; Oliveira, P.A.; Medeiros, R.; Vilanova, M.; da Gil Costa, R.M. Ptaquiloside from bracken (Pteridium spp.) inhibits tumour-infiltrating CD8+ T cells in HPV-16 transgenic mice. Food Chem. Toxicol. 2016, 97, 277–285.
25. da Gil Costa, R.M.; Lopes, C.; Oliveira, P.A.; Bastos, M.M.S.M. Illudane-Type Sesquiterpenes: Challenginges and Opportunities for Toxicology and Chemotherapy. In New Development in Terpene Research; Hu, J., Ed.; Nova Publishers, Inc.: New York, NY, USA, 2014; pp. 135–184. ISBN 978-1-62948-760-1.
26. Micheloud, J.F.; Caro, L.A.C.; Martínez, O.G.; Gimeno, E.J.; Ribeiro, D.D.S.F.; Blanco, B.S. Bovine enzootic haematuria from consumption of Pteris deflexa and Pteris plumula in northwestern Argentina. Toxicon 2017, 134, 26–29.
27. Rai, S.K.; Sharma, R.; Kumari, A.; Rasmussen, L.H.; Patil, R.D.; Bhar, R. Survey of ferns and clinico-pathological studies on the field cases of Enzootic bovine haematuria in Himachal Pradesh, a north-western Himalayan state of

India. Toxicon 2017, 138, 31–36.

28. Roperto, S.; Russo, V.; Ozkul, A.; Sepici-Dincel, A.; Maiolino, P.; Borzacchiello, G.; Marcus, I.; Esposito, I.; Riccardi, M.G.; Roperto, F. Bovine papillomavirus type 2 infects the urinary bladder of water buffalo (Bubalus bubalis) and plays a crucial role in bubaline urothelial carcinogenesis. J. Gen. Virol. 2013, 94, 403–408.
29. Roperto, F.; Russo, V.; Leonardi, L.; Martano, M.; Corrado, F.; Riccardi, M.G.; Roperto, F. Bovine Papillomavirus Type 13 Expression in the Urothelial Bladder Tumours of Cattle. Transbound. Emerg. Dis. 2016, 63, 628–634.
30. Roperto, S.; Munday, J.S.; Corrado, F.; Goria, M.; Roperto, F. Detection of bovine papillomavirus type 14 DNA sequences in urinary bladder tumors in cattle. Vet. Microbiol. 2016, 190, 1–4.
31. Gil Da Costa, R.M.; Medeiros, R. Bovine papillomavirus: Opening new trends for comparative pathology. Arch. Virol. 2013, 159, 191–198.
32. Corteggio, A.; Di Geronimo, O.; Roperto, S.; Roperto, F.; Borzacchiello, G. Activated platelet-derived growth factor β receptor and Ras–mitogen-activated protein kinase pathway in natural bovine urinary bladder carcinomas. Vet. J. 2012, 191, 393–395.
33. Gil Da Costa, R.M.; Peleteiro, M.C.; Pires, M.A.; DiMaio, D. An Update on Canine, Feline and Bovine Papillomaviruses. Transbound. Emerg. Dis. 2016, 64, 1371–1379.
34. Gil Da Costa, R.M.; Oliveira, P.A.; Vasconcelos-Nóbrega, C.; Arantes-Rodrigues, R.; Pinto-Leite, R.; Colaço, A.A.; De La Cruz, L.F.; Lopes, C. Altered expression of CKs 14/20 is an early event in a rat model of multistep bladder carcinogenesis. Int. J. Exp. Pathol. 2015, 96, 319–325.
35. Volkmer, J.-P.; Sahoo, D.; Chin, R.K.; Ho, P.L.; Tang, C.; Kurtova, A.V.; Willingham, S.B.; Pazhanisamy, S.K.; Contreras-Trujillo, H.; Storm, T.A.; et al. Three differentiation states risk-stratify bladder cancer into distinct subtypes. Proc. Natl. Acad. Sci. USA 2012, 109, 2078–2083.
36. Choi, W.; Czerniak, B.; Ochoa, A.; Su, X.; Siefker-Radtke, A.; Dinney, C.P.N.; McConkey, D.J. Intrinsic basal and luminal subtypes of muscle-invasive bladder cancer. Nat. Rev. Urol. 2014, 11, 400–410.
37. Shin, K.; Lim, A.; Odegaard, J.I.; Honeycutt, J.D.; Kawano, S.; Hsieh, M.H.; Beachy, P.A. Cellular origin of bladder neoplasia and tissue dynamics of its progression to invasive carcinoma. Nat. Cell Biol. 2014, 16, 469–478.
38. Robertson, A.G.; Kim, J.; Al-Ahmadie, H.; Bellmunt, J.; Guo, G.; Cherniack, A.D.; Hinoue, T.; Laird, P.W.; Hoadley, K.A.; Akbani, R.; et al. Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. Cell 2018, 174, 1033.
39. Shapiro, S.G.; Raghunath, S.; Williams, C.; Motsinger-Reif, A.; Cullen, J.M.; Liu, T.; Albertson, D.; Ruvolo, M.; Lucas, A.B.; Jin, J.; et al. Canine urothelial carcinoma: Genomically aberrant and comparatively relevant. Chromosom. Res. 2015, 23, 311–331.
40. John, B.A.; Said, N. Insights from animal models of bladder cancer: Recent advances, challenges, and opportunities. Oncotarget 2017, 8, 57766–57781.
41. Oliveira, P.A.; Vasconcelos-Nóbrega, C.; da Gil Costa, R.M.; Arantes-Rodrigues, R. The N-butyl-N-4-hydroxybutyl nitrosamine mouse urinary bladder cancer model. In Urothelial Carcinoma: Methods and Protocols; Schulz, W.A., Hoffmann, M.J., Niegisch, G., Eds.; Humana Press: New York, NY, USA, 2018; Volume 1655, pp. 155–167.
42. Carvalho, T.; Pinto, C.; Peleteiro, M. Urinary Bladder Lesions in Bovine Enzootic Haematuria. J. Comp. Pathol. 2006, 134, 336–346.
43. Cheng, L.; MacLennan, G.T.; Lopez-Beltran, A. Histologic grading of urothelial carcinoma: A reappraisal. Hum. Pathol. 2012, 43, 2097–2108.
44. Grassinger, J.M.; Merz, S.; Aupperle-Lellbach, H.; Erhard, H.; Klopfleisch, R. Correlation of BRAF Variant V595E, Breed, Histological Grade and Cyclooxygenase-2 Expression in Canine Transitional Cell Carcinomas. Vet. Sci. 2019, 6, 31.
45. Chun, R.; Garrett, L.D. Urogenital and mammary gland tumors. In Textbook of Veterinary Internal Medicine: Diseases of the Dog and the Cat, 7th ed.; Ettinger, S.J., Feldman, E.C., Eds.; Saunders Elsevier: St. Louis, MI, USA, 2010; pp. 2208–2212.
46. Heilmann, R.M.; Wright, Z.M.; Lanerie, D.J.; Suchodolski, J.S.; Steiner, J.M. Measurement of urinary canine S100A8/A9 and S100A12 concentrations as candidate biomarkers of lower urinary tract neoplasia in dogs. J. Vet. Diagn. Investig. 2014, 26, 104–112.
47. Cota, J.; Peleteiro, M.C.; Petti, L.; Tavares, L.; Duarte, A. Detection and quantification of bovine papillomavirus type 2 in urinary bladders and lymph nodes in cases of Bovine Enzootic Hematuria from the endemic region of Azores. Vet. Microbiol. 2015, 178, 138–143.

48. Borjesson, D.L.; DeJong, K. Urinary tract. In Canine and Feline Cytology: A Color Atlas and Interpretation Guide, 3rd ed.; Raskin, R.E., Meyer, D.J., Eds.; Saunders Elsevier: St. Louis, MI, USA, 2016; pp. 288–294.

49. Anderson, W.I.; Dunham, B.M.; King, J.M.; Scott, D.W. Presumptive subcutaneous surgical transplantation of a urinary bladder transitional cell carcinoma in a dog. Cornell Vet. 1989, 79, 263–266.

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

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

52. Higuchi, T.; Burcham, G.N.; Childress, M.O.; Rohleder, J.J.; Bonney, P.L.; Ramos-Vara, J.A.; Knapp, D.W. Characterization and treatment of transitional cell carcinoma of the abdominal wall in dogs: 24 cases (1985–2010). J. Am. Vet. Med. Assoc. 2013, 242, 499–506.

53. Iwasaki, R.; Shimosato, Y.; Yoshikawa, R.; Goto, S.; Yoshida, K.; Murakami, M.; Kawabe, M.; Sakai, H.; Mori, T. Survival analysis in dogs with urinary transitional cell carcinoma that underwent whole-body computed tomography at diagnosis. Vet. Comp. Oncol. 2019, 17, 385–393.

54. Owen, L.N.; World Health Organization. TNM Classification of tumours in domestic animals. In Veterinary Public Health Unit & WHO Collaborating Center for Comparative Oncology; World Health Organization: Geneva, Switzerland, 1980; 52p, Available online: http://www.who.int/iris/handle/10665/68618 (accessed on 18 November 2021).

55. Mochizuki, H.; Shapiro, S.G.; Breen, M. Detection of Copy Number Imbalance in Canine Urothelial Carcinoma with Droplet Digital Polymerase Chain Reaction. Vet. Pathol. 2015, 53, 764–772.

56. Vinall, R.L.; Kent, M.S.; White, R.W.D. Expression of microRNAs in urinary bladder samples obtained from dogs with grossly normal bladders, inflammatory bladder disease, or transitional cell carcinoma. Am. J. Vet. Res. 2012, 73, 1626–1633.

57. Zhang, J.; Wei, S.; Liu, L.; Gowda, G.N.; Bonney, P.; Stewart, J.; Knapp, D.W.; Raftery, D. NMR-based metabolomics study of canine bladder cancer. Biochim. Biophys. Acta (BBA) Mol. Basis Dis. 2012, 1822, 1807–1814.

58. Decker, B.; Parker, H.G.; Dhawan, D.; Kwon, E.M.; Karlins, E.; Davis, B.W.; Ramos-Vara, J.A.; Bonney, P.L.; McNiel, E.A.; Knapp, D.W.; et al. Homologous Mutation to Human BRAF V600E Is Common in Naturally Occurring Canine Bladder Cancer—Evidence for a Relevant Model System and Urine-Based Diagnostic Test. Mol. Cancer Res. 2015, 13, 993–1002.

59. Mochizuki, H.; Kennedy, K.; Shapiro, S.G.; Breen, M. BRAF Mutations in Canine Cancers. PLoS ONE 2015, 10, e0129534.

60. Mochizuki, H.; Shapiro, S.G.; Breen, M. Detection of BRAF Mutation in Urine DNA as a Molecular Diagnostic for Canine Urothelial and Prostatic Carcinoma. PLoS ONE 2015, 10, e0144170.

61. Parker, H.G.; Dhawan, D.; Harris, A.C.; Ramos-Vara, J.A.; Davis, B.W.; Knapp, D.W.; Ostrander, E.A. RNAseq expression patterns of canine invasive urothelial carcinoma reveal two distinct tumor clusters and shared regions of dysregulation with human bladder tumors. BMC Cancer 2020, 20, 251.

62. Gedon, J.; Kehl, A.; Aupperle-Lellbach, H.; von Bomhard, W.; Schmidt, J.M. BRAF mutation status and its prognostic significance in 79 canine urothelial carcinomas: A retrospective study (2006–2019). Vet. Comp. Oncol. 2021.

63. Tagawa, M.; Tambo, N.; Maezawa, M.; Tomihari, M.; Watanabe, K.-I.; Inokuma, H.; Miyahara, K. Quantitative analysis of the BRAF V595E mutation in plasma cell-free DNA from dogs with urothelial carcinoma. PLoS ONE 2020, 15, e0232365.

64. Antech Diagnostics. CADET® BRAF and CADET® BRAF PLUS ; Antech Diagnostics, Inc.: Fountain Valley, CA, USA, 2021; Available online: https://www.antechdiagnostics.com/laboratory-diagnostics/molecular-diagnostics/cadet-braf-plus (accessed on 14 November 2021).

65. Rossman, P.; Zabka, T.S.; Ruple, A.; Tuerck, D.; Ramos-Vara, J.A.; Liu, L.; Mohallem, R.; Merchant, M.; Franco, J.; Fulkerson, C.M.; et al. Phase I/II Trial of Vemurafenib in Dogs with Naturally Occurring, BRAF-mutated Urothelial Carcinoma. Mol. Cancer Ther. 2021, 20, 2177–2188.

66. Marconato, L.; Sabattini, S.; Marisi, G.; Rossi, F.; Leone, V.F.; Casadei-Gardini, A. Sorafenib for the Treatment of Unresectable Hepatocellular Carcinoma: Preliminary Toxicity and Activity Data in Dogs. Cancers 2020, 12, 1272.

67. Jung, H.; Bae, K.; Lee, J.Y.; Kim, J.-H.; Han, H.-J.; Yoon, H.-Y.; Yoon, K.-A. Establishment of Canine Transitional Cell Carcinoma Cell Lines Harboring BRAF V595E Mutation as a Therapeutic Target. Int. J. Mol. Sci. 2021, 22, 9151.

68. Henry, C.J.; Tyler, J.W.; McEntee, M.C.; Stokol, T.; Rogers, K.S.; Chun, R.; Garrett, L.D.; McCaw, D.L.; Higginbotham, M.L.; Flessland, K.A.; et al. Evaluation of a bladder tumor antigen test as a screening test for transitional cell carcinoma of the lower urinary tract in dogs. Am. J. Vet. Res. 2003, 64, 1017–1020.

69. Polymedco. V-BTA Test; Package Insert on the Internet; Polymedco, Inc.: Cortland Manor, NY, USA, 2010; Available online: https://www.vetbta.com/veterinarians (accessed on 14 November 2021).

70. Borjesson, D.L.; Christopher, M.M.; Ling, G.V. Detection of canine transitional cell carcinoma using a bladder tumor antigen urine dipstick test. Vet. Clin. Pathol. 1999, 28, 33–38.

71. Billet, J.-P.H.; Moore, A.H.; Holt, P.E. Evaluation of a bladder tumor antigen test for the diagnosis of lower urinary tract malignancies in dogs. Am. J. Vet. Res. 2002, 63, 370–373.

72. Sotirakopoulos, A.; Armstrong, P.; Heath, L.; Madrill, N.; McNiel, E. Evaluation of Microsatellite Instability in Urine for the Diagnosis of Transitional Cell Carcinoma of the Lower Urinary Tract in Dogs. J. Vet. Intern. Med. 2010, 24, 1445–1451.

73. Allen, D.K.; Waters, D.J.; Knapp, D.W.; Kuczek, T. High Urine Concentrations of Basic Fibroblast Growth Factor in Dogs with Bladder Cancer. J. Vet. Intern. Med. 1996, 10, 231–234.

74. R&D Systems. Quantikine® HS ELISA—Human FGF Basic Immunoassay; Package Insert on the Internet; R&D Systems, Inc.: Minneapolis, MN, USA, 2020; Available online: https://www.rndsystems.com/products/human-fgf-basic-quantikine-hs-elisa-kit_hsfb00d (accessed on 18 November 2021).

75. Creative Diagnostics. Canine Basic Fibroblast Growth Factor ELISA Kit; Package Insert on the Internet; Creative Diagnostics: Shirley, NY, USA, 2021; Available online: https://www.creative-diagnostics.com/FGF2-EIA-Kit-246601-463.htm (accessed on 18 November 2021).

76. Genorise Scientific, Inc. Nori® Canine FGF Basic ELISA Kit—DataSheet; Package Insert on the Internet; Genorise Scientific, Inc.: Glen Mills, PA, USA, 2021; Available online: http://www.genorise.com/Canine-FGF-ELISA-Kits.html (accessed on 18 November 2021).

77. Mohammed, S.I.; Bennett, P.F.; Craig, B.A.; Glickman, N.W.; Mutsaers, A.J.; Snyder, P.W.; Widmer, W.R.; de Gortari, A.E.; Bonney, P.L.; Knapp, D.W. Effects of the cyclooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. Cancer Res. 2002, 15, 356–358. Available online: http://cancerres.aacrjournals.org/content/62/2/356.abstract (accessed on 23 September 2020).

78. Mohammed, S.I.; Craig, B.A.; Mutsaers, A.J.; Glickman, N.W.; Snyder, P.W.; De Gortari, A.E.; Schlittler, D.L.; Coffman, K.T.; Bonney, P.L.; Knapp, D.W. 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. 2003, 2, 183–188. Available online: https://mct.aacrjournals.org/content/2/2/183 (accessed on 23 September 2020).

79. Abbott Laboratories. UroVysion Bladder Cancer Kit; Package Insert on the Internet; Abbott Molecular, Inc.: Des Plaines, IL, USA, 2021; Available online: https://www.molecular.abbott/us/en/products/oncology/urovysion-bladder-cancer-kit (accessed on 18 November 2021).

80. Kent, M.S.; Zwingenberger, A.; Westropp, J.L.; Barrett, L.E.; Durbin-Johnson, B.P.; Ghosh, P.; Vinall, R.L. MicroRNA profiling of dogs with transitional cell carcinoma of the bladder using blood and urine samples. BMC Vet. Res. 2017, 13, 339.

81. Kim, N.W.; Piatyszek, M.A.; Prowse, K.R.; Harley, C.B.; West, M.D.; Ho, P.D.L.; Coviello, G.M.; Wright, W.E.; Weinrich, S.L.; Shay, J.W. Specific association of human telomerase activity with immortal cells and cancer. Science 1994, 266, 2011–2015.

82. McCleary-Wheeler, A.L.; Williams, L.E.; Hess, P.R.; Suter, S.E. Evaluation of an in vitro telomeric repeat amplification protocol assay to detect telomerase activity in canine urine. Am. J. Vet. Res. 2010, 71, 1468–1474.

83. Heilmann, R.M.; McNiel, E.A.; Grützner, N.; Lanerie, D.J.; Suchodolski, J.S.; Steiner, J.M. Diagnostic performance of the urinary canine calgranulins in dogs with lower urinary or urogenital tract carcinoma. BMC Vet. Res. 2017, 13, 112.

84. Bracha, S.; McNamara, M.; Hilgart, I.; Milovancev, M.; Medlock, J.; Goodall, C.; Wickramasekara, S.; Maier, C.S. A multiplex biomarker approach for the diagnosis of transitional cell carcinoma from canine urine. Anal. Biochem. 2014, 455, 41–47.

85. Dill, A.L.; Ifa, D.R.; Manicke, N.E.; Costa, A.B.; Ramos-Vara, J.A.; Knapp, D.W.; Cooks, R.G. 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. 2009, 81, 8758–8764.

86. Pope, G.B. Urinary Lipid Biomarkers for Detecting Canine Transitional Cell Carcinoma Pilot Study. Bachelor's Thesis, Oregon State University, Corvallis, OR, USA, 2016. Available online:

http://ir.library.oregonstate.edu/concern/undergraduate_thesis_or_projects/bn999c19c (accessed on 20 September 2020).

87. Rankin, W.V.; Henry, C.J.; Turnquist, S.E.; Turk, J.R.; Beissenherz, M.E.; Tyler, J.W.; Green, J.A. Comparison of distributions of survivin among tissues from urinary bladders of dogs with cystitis, transitional cell carcinoma, or histologically normal urinary bladders. Am. J. Vet. Res. 2008, 69, 1073–1078.
88. Hanazono, K.; Fukumoto, S.; Kawamura, Y.; Endo, Y.; Kadosawa, T.; Iwano, H.; Uchide, T. Epidermal growth factor receptor expression in canine transitional cell carcinoma. J. Vet. Med. Sci. 2015, 77, 1–6.
89. Millanta, F.; Impellizeri, J.; McSherry, L.; Rocchigiani, G.; Aurisicchio, L.; Lubas, G. Overexpression of HER-2 via immunohistochemistry in canine urinary bladder transitional cell carcinoma—A marker of malignancy and possible therapeutic target. Vet. Comp. Oncol. 2017, 16, 297–300.
90. Walters, L.; Martin, O.; Price, J.; Sula, M.M. Expression of receptor tyrosine kinase targets PDGFR-β, VEGFR2 and KIT in canine transitional cell carcinoma. Vet. Comp. Oncol. 2017, 16, E117–E122.
91. Korec, D.I.; Louke, D.S.; Breitbach, J.T.; Geisler, J.A.; Husbands, B.D.; Fenger, J.M. Characterization of receptor tyrosine kinase activation and biological activity of toceranib phosphate in canine urothelial carcinoma cell lines. BMC Vet. Res. 2021, 17, 320.
92. London, C.A.; Hannah, A.L.; Zadovoskaya, R.; Chien, M.B.; Kollias-Baker, C.; Rosenberg, M.; Downing, S.; Post, G.; Boucher, J.; Shenoy, N.; et al. Phase I dose-escalating study of SU11654, a small molecule receptor tyrosine kinase inhibitor, in dogs with spontaneous malignancies. Clin. Cancer Res. 2003, 9, 2755–2768.
93. Rippy, S.B.; Gardner, H.L.; Nguyen, S.M.; Warry, E.E.; Portela, R.A.; Drost, W.T.; Hostnik, E.T.; Green, E.M.; Chew, D.J.; Peng, J.; et al. A pilot study of toceranib/vinblastine therapy for canine transitional cell carcinoma. BMC Vet. Res. 2016, 12, 257.
94. Gustafson, T.L.; Biller, B. Use of Toceranib Phosphate in the Treatment of Canine Bladder Tumors: 37 Cases. J. Am. Anim. Hosp. Assoc. 2019, 55, 243–248.
95. Inoue, A.; Maeda, S.; Kinoshita, R.; Tsuboi, M.; Yonezawa, T.; Matsuki, N. Density of tumor-infiltrating granzyme B-positive cells predicts favorable prognosis in dogs with transitional cell carcinoma. Vet. Immunol. Immunopathol. 2017, 190, 53–56.
96. Hanazono, K.; Nishimori, T.; Fukumoto, S.; Kawamura, Y.; Endo, Y.; Kadosawa, T.; Uchide, T. Immunohistochemical expression of p63, Ki67 andβ-catenin in canine transitional cell carcinoma and polypoid cystitis of the urinary bladder. Vet. Comp. Oncol. 2016, 14, 263–269.
97. Barbareschi, M.; Pecciarini, L.; Cangi, M.G.; Macrì, E.; Rizzo, A.; Viale, G.; Doglioni, C. p63, a p53 Homologue, Is a Selective Nuclear Marker of Myoepithelial Cells of the Human Breast. Am. J. Surg. Pathol. 2001, 25, 1054–1060.
98. Signoretti, S.; Waltregny, D.; Dilks, J.; Isaac, B.; Lin, D.; Garraway, L.; Yang, A.; Montironi, R.; McKeon, F.; Loda, M. p63 Is a Prostate Basal Cell Marker and Is Required for Prostate Development. Am. J. Pathol. 2000, 157, 1769–1775.
99. Di Como, C.J.; Urist, M.J.; Babayan, I.; Drobnjak, M.; Hedvat, C.V.; Teruya-Feldstein, J.; Pohar, K.; Hoos, A.; Cordon-Cardo, C. p63 expression profiles in human normal and tumor tissues. Clin. Cancer Res. 2002, 8, 494–501. Available online: http://clincancerres.aacrjournals.org/content/8/2/494.long (accessed on 20 September 2018).
100. Urist, M.J.; DiComo, C.; Lu, M.-L.; Charytonowicz, E.; Verbel, D.; Crum, C.P.; Ince, T.A.; McKeon, F.D.; Cordon-Cardo, C. Loss of p63 Expression Is Associated with Tumor Progression in Bladder Cancer. Am. J. Pathol. 2002, 161, 1199–1206.
101. Koga, F.; Kawakami, S.; Fujii, Y.; Saito, K.; Ohtsuka, Y.; Iwai, A.; Ando, N.; Takizawa, T.; Kageyama, Y.; Kihara, K. Impaired p63 expression associates with poor prognosis and uroplakin III expression in invasive urothelial carcinoma of the bladder. Clin. Cancer Res. 2003, 9, 5501–5507.
102. Nishimori, T.; Hanazono, K.; Matsuda, K.; Kawamura, Y.; Kadosawa, T.; Endo, Y.; Uchide, T. Prognostic role of and ΔNp63 expression in canine transitional cell carcinoma of the urinary bladder. Open Vet. J. 2021, 11, 700.
103. Wu, X.; Lin, J.; Walz, T.; Häner, M.; Yu, J.; Aebi, U.; Sun, T. Mammalian uroplakins. A group of highly conserved urothelial differentiation-related membrane proteins. J. Biol. Chem. 1994, 269, 13716–13724.
104. Ramos-Vara, J.A.; Miller, M.A.; Boucher, M.; Roudabush, A.; Johnson, G.C. Immunohistochemical Detection of Uroplakin III, Cytokeratin 7, and Cytokeratin 20 in Canine Urothelial Tumors. Vet. Pathol. 2003, 40, 55–62.
105. Sledge, D.G.; Patrick, D.J.; Fitzgerald, S.D.; Xie, Y.; Kiupel, M. Differences in Expression of Uroplakin III, Cytokeratin 7, and Cyclooxygenase-2 in Canine Proliferative Urothelial Lesions of the Urinary Bladder. Vet. Pathol. 2014, 52, 74–82.