Early Detection of Urothelial Carcinoma and Juvenile-Onset Nephropathy

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What Is Urothelial Carcinoma?

Urothelial carcinoma (UC), also referred to as transitional cell carcinoma (TCC), is a common cancer of the urinary tract that is both challenging to diagnose and treat effectively. This cancer represents 1-2% of all canine cancers, affecting 60,000-120,000 dogs per year. Westies in particular are 3-6x more likely to develop this cancer than the general dog population. In other words, 1 in 25 Westies aged 6 or older will develop UC/TCC. Typically, UC/TCC is not suspected until a dog develops clinical signs or urinary discomfort, such as bloody urine, straining to urinate, accidents in the house, and/or frequent urination. Diagnosis involves obtaining samples to identify the cancer as UC/TCC and to evaluate the location of any lesions. UC/TCC typically involves the bladder, urethra, and/or prostate. The current gold standard for UC/TCC diagnosis is via tissue biopsy, typically through cystoscopy. Abdominal imaging, such as ultrasound, and cystoscopy can be used to determine where the tumor is located. Distant metastases (spreading of UC/TCC to other organs such as lymph nodes, lungs, and the liver) are present in ~20% of dogs with UC/TCC at the time of diagnosis. Therefore, imaging such as abdominal ultrasound, thoracic radiographs, and/or CT scan can also be used to look for evidence of metastases. Once diagnosed, treatment options include chemotherapy, radiation therapy, and surgery. Currently, at the time of diagnosis over 90% of cases of canine TCC/UC are of intermediate to high-grade and invasive. Due to its aggressive nature, UC/TCC treatment is rarely curative, and ultimately the tumor progresses. The ability to diagnose UC/TCC earlier in its disease course could have a powerful impact on blunting its progression.

The NC State College of Veterinary Medicine Urothelial Carcinoma Research Group is evaluating a newly developed test, the CADET® *BRAF*, to see if it is capable of early detection of urothelial carcinoma. Early, noninvasive diagnosis could be a game-changer in improving survival of dogs with UC/TCC. Additionally, we are investigating environmental exposure to chemicals, such as pesticides and flame retardants, in both dogs with early bladder cancer and healthy dogs.

What Is The CADET® *BRAF?*

The foundation for the CADET® *BRAF* comes from two recent studies performed by research teams at North Carolina State University (NC State) and the National Institutes of Health (NIH). A single mutation in the canine *BRAF* gene was detected in pathology-verified tumor biopsy specimens of canine UC/TCC. The result of this single mutation is one amino acid change (valine to glutamic acid) in the *BRAF* protein in the tumor cells. This change results in a mutated protein that signals the cells to proliferate, leading

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to the development of a tumor. The *BRAF* mutation has not been detected in numerous non-neoplastic bladder tissues, including inflammatory bladder tissue and polyps.

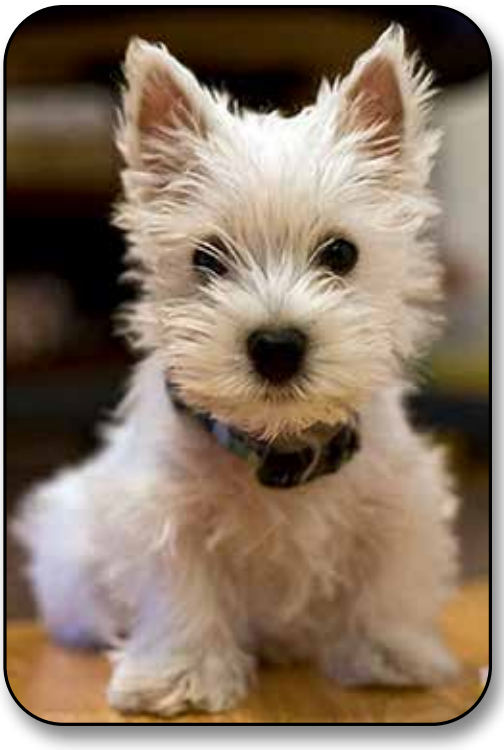
In cases where a dog has a UC/TCC, cells from the mass, ranging from very early to late in the course of disease, are shed into the urine and so contribute to the cells recovered. The Breen Lab at NC State developed a rapid and highly sensitive test to detect the presence of this mutation in cells shed into the urine. Further development by Sentinel Biomedical *(www.SentinelBiomedical.com)* led to the refinement and commercialization of the world's first liquid biopsy for a veterinary cancer in the form of the CADET® *BRAF*. Quantification is presented as a percent fractional abundance of mutant alleles. Most cells with the *BRAF* mutation are heterozygous, so the fractional abundance of the mutation typically does not exceed 50%.

The CADET® *BRAF* can identify 85% of canine UC/TCC. In all cases that have had a biopsy of a visible mass for pathology evaluation, there is 100% concordance between the presence of a *BRAF* mutation detected in free-catch urine and subsequent confirmation of a UC/TCC in the biopsy of the mass. In contrast, a *BRAF* mutation has not been detected in urine specimens from dogs that were shown not to have a TCC/UC. Unlike previous tests for canine TCC/UC, CADET® *BRAF* blood or bacteria in the urine does not affect results. The test can detect the *BRAF* mutation in as few as 10 cells shed into the urine, and therefore provides a powerful opportunity to use this mutation as a screening test to diagnose UC/TCC earlier. However, the CADET® *BRAF* has not been fully evaluated in asymptomatic dogs.

Screening Healthy Westies For UC/TCC

This clinical study at NC State, called the Early Detection of Urothelial Carcinoma Clinical Trial, involves screening healthy dogs over the age of 6 years from several AKC-registered breeds at an increased risk of developing UC/TCC. These breeds include West Highland White Terriers, Scottish Terriers, Beagles, Shetland Sheepdogs, Russell/Parson Russell Terriers, and American Eskimo dogs. As with all dogs affected by UC/TCC, the majority of tumors in the Westie are detected in the bladder.

Potential participants are asked to complete an online questionnaire and consent form. Upon completion, each dog owner will be sent a urine collection kit, which is very easy to use. Owners collect free catch urine from their dog at home in a clean household container and pour it into the collection pot provided. The pot is then shipped back to the lab to be tested.



All packing materials and prepaid FedEx shipping labels are included with each kit. On receipt at NC State Veterinary School, urine samples will be analyzed by CADET® *BRAF* to identify dogs that are shedding a very low level of *BRAF* mutant cells into their urine, indicative of the presence of an early TCC/UC. We plan on screening over 1000 dogs across the country.

From the >1,000 dogs screened, 30 dogs with a low urinary *BRAF* mutation level will have a detailed clinical evaluation through physical examination, blood tests, urinalysis, ultrasound, cystoscopy, and biopsy of any visible lesions. The goal of the clinical evaluation is to obtain samples for definitive diagnosis and to determine the extent and location of any suspected UC/TCC. For all 30 dogs, the level of *BRAF* mutation shed into the urine will be monitored monthly. Once the levels reach 10%, dogs will have repeat diagnostics, including ultrasound, urine cytology, and cystoscopy with biopsy. The cost of all provided clinical evaluations and associated CADET® *BRAF* testing will be covered by the study. Participation will end when a definitive diagnosis of UC/TCC is made through urine cytology or histopathology of tumor biopsies.

If the CADET® *BRAF* is proven as an effective screening test for UC/TCC, earlier diagnosis prior to the development of clinical signs holds promise for improved survival and quality of life for dogs diagnosed with this devastating cancer. Through periodic monitoring, this study also aims to determine the time period from detection of a low level of the *BRAF* mutation to the time that the dog shows clinical signs of UC/TCC. This interval may help guide future recommendations on the frequency of screening as well.

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If you are interested in getting involved, please complete this questionnaire: https://is.gd/NCStateUCClinicalTrial

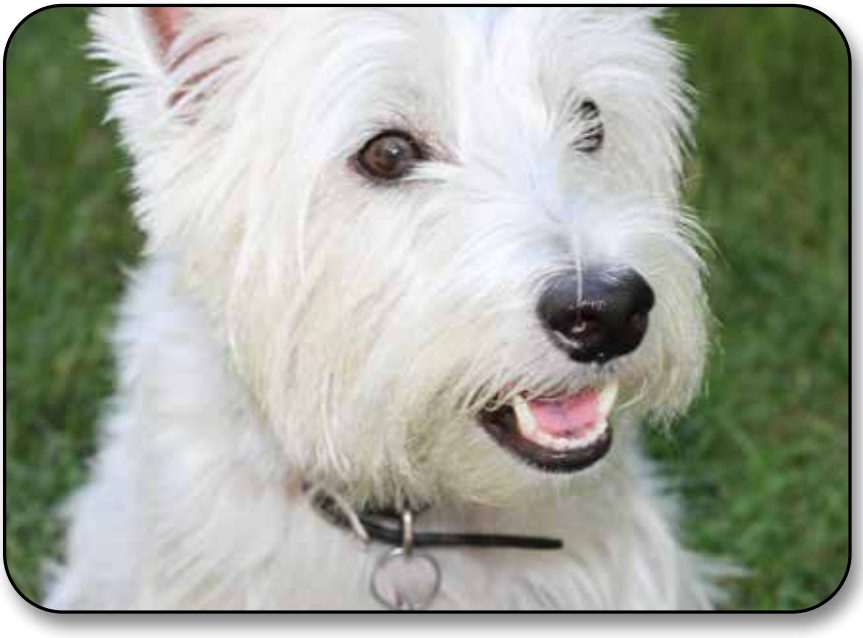
What Do We Recommend For Westies?

Although the study is ongoing, preliminary results suggests that screening asymptomatic dogs using the CADET® *BRAF* may aid in identifying UC/TCC 3-6 months or longer before clinical signs develop. Diagnosing UC/TCC earlier in the course of disease provides more time for the dog to be treated for this cancer. However, more studies are necessary to see if earlier treatment leads to increased survival. Starting at six years of age, we recommend using the CADET® *BRAF* to screen the urine of Westies every 4-6 months.

JUVENILE-ONSET NEPHROPATHY

What Is Juvenile-Onset Nephropathy?

Juvenile onset nephropathy, also known as renal dysplasia or juvenile dysplasia, represents a group of developmental anomalies defined as an abnormal differentiation of the



kidneys. Renal disease of any kind can be challenging to diagnose and treat early. Kidneys have incredible redundancy, and signs related to kidney disease do not develop until greater than 66% of both kidneys combined are damaged. Increased drinking (polydipsia) and urination (polyuria) develop once greater than 66% of the kidneys are damaged, and increased BUN and creatinine (azotemia) develop once greater than 75% of the kidneys are damaged. Other clinical signs related to kidney disease include vomiting, nausea, decrease appetite, weight loss, and muscle-wasting.

Juvenile-onset nephropathy often presents similar to chronic kidney disease, with these clinical signs gradually developing as kidney function declines. Therefore, juvenile-onset nephropathy can have a highly variable age of onset. **This late diagnosis means that dogs with juvenile-onset nephropathy could be bred before they show any clinical signs.**

Juvenile-onset nephropathy affects multiple breeds, including some terrier breeds related to Westies (Soft-coated Wheaten terriers, Cairn terriers). The disease is well characterized in some breeds, such as Lhasa Apso and Shih Tzu, and poorly characterized in others, often with only a single case or litter documented. Other breeds are likely affected.

How Is Juvenile-Onset Nephropathy Diagnosed And Treated?

Like many kidney diseases, juvenile-onset nephropathy is often diagnosed late in the disease course once clinical signs develop. Affected kidneys appear small and scarred, similar to those with advanced chronic kidney disease (CKD). Diagnosis is made with biopsy and histopathology. A large sample obtained through a surgical wedge biopsy is recommended. Because juvenile-onset nephropathy is a group of diseases, histopathology can be varied. The most consistent abnormality is inappropriate differentiation of nephron components, such as the presence of immature nephrons along with normal nephrons. Because the immature nephrons cannot function appropriately, the normal nephrons try to compensate and become hypertrophied. Histopathology may also show signs of acquired damage, such as inflammation, scarring, and mineralization. This acquired damage can make diagnosis of juvenile-onset nephropathy very challenging. If enough damage is present, then the immature nephrons or other diagnostic features may not be visible. **In other words, kidneys from a dog that died of juvenile-onset nephropathy could be identical to kidneys from a dog that died of CKD, even on histopathology.**

Although biopsies are imperative for diagnosis, ultrasound shows promise in identifying preclinical juvenile-onset nephropathy, and therefore aid in determining which dogs to obtain biopsies from. A study evaluating ultrasonographic findings in Cairn terriers showed that the severity of ultrasonographic disease observed was comparable to the

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severity of the histopathologic abnormalities, even in dogs as young as 4 months old. Kidneys with juvenile-onset nephropathy can look identical to those with other kidney diseases, so biopsy is always recommended. Treatment for juvenile-onset nephropathy involves supportive care, similar to that required for CKD. However, biopsy of the kidneys could identify a different type of kidney disease, and therefore necessitate different treatments.

What About Westies?

Although Westies have not been listed as an at-risk breed, the difficulty in diagnosing juvenile-onset nephropathy makes it very possible that other breeds, including Westies, are affected. Breeds related to Westies, including Cairns and soft-coated Wheaten terriers, also have reported cases of juvenile-onset nephropathy. More information is necessary to determine if this kidney disease should be a concern to breeders.

Moving forward, I recommend the club starts by discussing their goals in investigating juvenile-onset nephropathy. A starting point could be discussing the prevalence of kidney disease of any kind in Westies, because juvenile-onset nephropathy can appear similar to CKD. Once the scope of possible disease is established, the club can discuss additional recommendations. A starting point could be obtaining kidney samples from dogs that die of CKD. For consistency we recommend sending kidney samples to the International Veterinary Renal Pathology Service, a collaborative effort between the Ohio State University and Texas A&M University. Additionally, a database of all cases of juvenile-onset nephropathy could be established. Another consideration would be to start performing ultrasounds on a few litters when the puppies are 4 months of age to see if any abnormalities are detected. Ultimately, no one is more dedicated to upholding the health of Westies than their breeders and owners, and so next steps should reflect a collaboration between the Westie club and veterinarians.

References:

Decker B, Parker HG, Dhawan D, Kwon EM, Karlins E, Davis BW, 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(6):993-1002. doi: 10.1158/1541-7786.MCR-14- 0689. PubMed PMID: 25767210; PubMed Central PMCID: PMC4470794.

Fulkerson CM, Knapp DW. Management of transitional cell carcinoma of the urinary bladder in dogs: a review. Vet J. 2015;205(2):217-225.

Knapp D, McMillan S. Tumors of the urinary system. In: Withrow SJ VD, ed. *Withrow and MacEwen's Small Animal Clinical Oncology*. . Vol 5th ed. . St. Louis: Elsevier–Saunders; 2013:572-582.

Knapp DW, Ramos-Vara JA, Moore GE, Dhawan D, Bonney PL, Young KE. Urinary bladder cancer in dogs, a naturally occurring model for cancer biology and drug development. *ILAR J*. 2014;55(1):100-118.

Marvel SJ, Seguin B, Dailey DD, Thamm DH. Clinical outcome of partial cystectomy for transitional cell carcinoma of the canine bladder. *Vet Comp Oncol*. 2017;15(4):1417-1427.

Mochizuki H, Kennedy K, Shapiro SG, Breen M. *BRAF* Mutations in Canine Cancers. PLoS One. 2015;10(6):e0129534. doi: 10.1371/journal.pone.0129534.

Mochizuki H, Shapiro SG, Breen M. Detection of *BRAF* Mutation in Urine DNA as a Molecular Diagnostic for Canine Urothelial and Prostatic Carcinoma. PLoS One. 2015;10(12):e0144170

Nolan MW, Kogan L, Griffin LR, et al. Intensity-modulated and image-guided radiation therapy for treatment of genitourinary carcinomas in dogs. *J Vet Intern Med*. 2012;26(4):987-995.

Picut CA, LewisRM. Microscopic features of canine renal dysplasia. *Vet Pathol*. 1987;24:156-163.

Seiler GS et al. Ultrasonographic findings in Cairn Terriers with preclinical renal dysplasia. *Vet Radiol Ultrasound*. 2010;51(4):453- 457