

Canine Transitional Cell Carcinoma (TCC) / Urothelial Carcinoma (UC)/Bladder Cancer in Dogs

New research provides an opportunity for early detection in the West Highland White Terrier.

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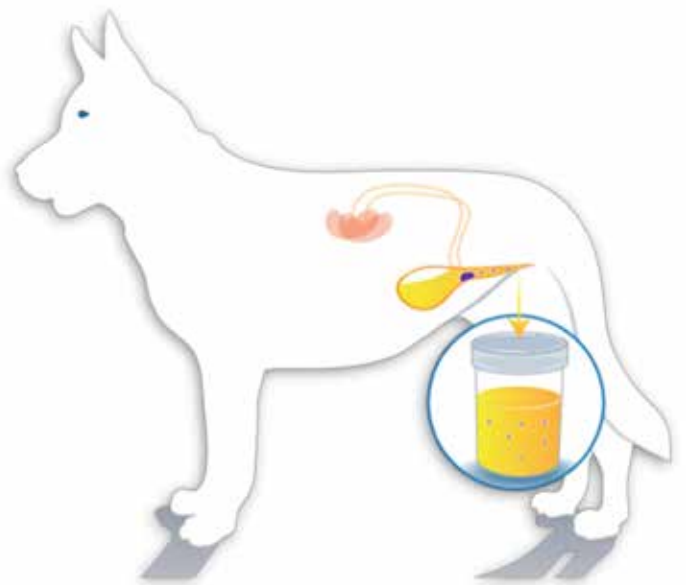
Canine TCC/UC/Bladder Cancer.

Canine transitional cell carcinoma (TCC), also known as urothelial carcinoma (UC), is the most common cancer of the canine urinary tract (1). Across all breeds the cancer represents an estimated 1-2% of all canine cancer, and with over 6 million cancers diagnosed in pet dogs each year in the US, the number of canine TCCs/UCs is therefore estimated to be 60,000-120,000. However, a group of breeds, including the West Highland White Terrier, have a much higher chance of developing the cancer. TCC/UC is generally a disease of mid to late life, with over 90% of cases occurring in dogs age 6 years and older. TCC/UC affects the bladder, urethra, and kidneys of male and female dogs, and also the prostate of males. Clinical presentation of advancing TCC/UC is shared with other much more common urinary tract disorders, including cystitis and prostatitis. These may include one or more of the following: straining to urinate; repeated frequent attempts to urinate; blood in the urine; and bacterial infection.

TCC/UC is most often detected in the trigone of the bladder, a triangular region of smooth mucosa inside the dorsal wall of the neck of the bladder. Any thickening of the bladder wall in this location can lead to partial or complete obstruction of urine entering the bladder from the ureters, which may lead to kidney failure, or exiting the bladder through the urethra (2).

How Is TCC/UC Currently Diagnosed?

A common route to diagnosis of a TCC/UC is one in which the dog is first taken to a veterinarian to assess the likely cause of the urinary tract symptoms stated above. In most cases the dog is then treated for the above symptoms, on the assumption that there is a non-malignant cause. It is common for these symptoms to initially be managed with repeated cycles of urine culture followed with antibiotic administration, and sometimes anti-inflammatory medications over several months. While this treatment approach may provide temporary relief of the symptoms, the underlying cancer causing these symptoms is still progressing. Consequently, during the time that the dog is being treated for the symptoms, the tumor can develop into a more advanced state, becoming larger, potentially invading the muscle



wall, and also having a greater chance of spreading to other parts of the dog's body (metastasis). When repeated treatments for the symptoms fail to fully resolve them, other conditions are considered and the dog is then evaluated for the presence of a TCC/UC, usually via urine cytology, abdominal ultrasound, and/or cystoscopy.

Where a mass is detected, it is recommended that a biopsy be taken and submitted for a histopathology evaluation, which serves to confirm the diagnosis of a TCC/UC and may also indicate if the mass has invaded the muscle wall. Further imaging and evaluation of local lymph nodes may be performed to assess the spread of the disease. Currently, at the time of diagnosis over 90% of cases of canine TCC/UC are of intermediate to high-grade and invasive (3). Superficial, low-grade tumors are very uncommon. In addition, at the time of diagnosis, ~20% of canine TCCs/UCs have already spread to other parts of the body (2).

The high predominance of advanced tumors detected by conventional means may reflect the prolonged time taken to diagnose the tumors in most cases.

(Continued on page 16)



How Is TCC/UC Currently Treated?

Currently, once finally diagnosed, treatment of canine TCC/UC most commonly includes the use of chemotherapy, cyclooxygenase inhibitors, and combinations of these drugs. Where single agent therapy is used, the proportion of dogs entering remission is generally low (<20%), although this is increased to 35–50% with combined chemotherapy and cyclooxygenase inhibitors. While less common than drug based intervention, surgery and radiation therapy are also used where appropriate (1). Regardless of the common drug treatment option used, median survival of treated dogs with TCC/UC is currently ~7-9 months.

What Is The Challenge?

Finding abnormal epithelial cells in urine sediment, or in samples obtained by traumatic catheterization, prostatic wash, and/or fine needle aspiration is used to support the diagnosis of canine TCC/UC (1,4,5). Cytological analysis of epithelial cells, however, may be misleading. For example, benign epithelial cells can resemble malignant cells with variation in cell size, and an increased number of basophils may be present after prolonged contact with urine or secondary to an inflammatory condition (6). Fine needle aspiration of tumor tissue carries the risk of disseminating tumor cells along the needle tract and so should be performed with caution (7,8). Currently, clinical diagnosis of canine TCC/UC requires comprehensive diagnostic workups, including blood test, urinalysis and diagnostic imaging, in

addition to cytological examinations of tumor cells by skilled clinical pathologists, and histopathology of a biopsy specimen.

Regardless of the diagnostic process used, most TCCs/UCs currently go undiagnosed until they are at an advanced clinical stage and so are associated with guarded-poor prognosis. Improved methods for earlier and less invasive detection are needed. Detection of the presence of a TCC/UC earlier in the course of disease would allow appropriate intervention sooner, which is expected to improve quality of life and extend survival. At the very least, earlier identification of the presence of a TCC/UC as the underlying cause of the common symptoms would avoid prolonged delays in detecting the cancer, expediting treating of the cancer and not just the symptoms.

The availability of a reliable, non-invasive diagnostic test for canine UC/TCC is a paramount need, as is the availability of a means to reliably detect the presence of a TCC/UC early in the course of the disease.

What Is The New Opportunity For Early Detection Of Canine TCC/UC?

In two recent independent studies, performed by research teams at North Carolina State University (NCSU) (9) and the National Institutes of Health (NIH) (10), a single mutation in the canine BRAF gene was detected in pathology verified tumor biopsy specimens of canine TCC/UC. The NCSU team identified the mutation comparing the DNA sequences of all genes of the dog DNA isolated from TCC/UC tissue samples with those from non-neoplastic tissues. The NIH team identified the mutation by looking at RNA sequences in affected tissues. The discovery of the same mutation independently by two groups using two different approaches provides cross validation of the data.

***TECHNICAL DETAILS.** In the canine genome sequence at nucleotide position 8,296,284 on dog chromosome 16, the DNA nucleotide is a "T", but in the tumor cells of 85% of cases of TCC/UC, this base has mutated to an "A". The result of this single mutation is one amino change in the BRAF protein; the amino acid is supposed to be a valine, but in the tumor cells it is a glutamic acid. This mutation is located in the activation segment of the kinase domain of the BRAF gene and the change in this one amino acid produces a mutated protein with increased kinase activity. The consequence of this change is that it signals the cells to proliferate, leading to the development of a tumor.*

The NCSU team showed that the BRAF mutation was not present in numerous other canine cancers and in non-neoplastic bladder tissues, including inflammatory bladder tissue and

(Continued on page 17)

(TCC/UC/Bladder Cancer in Dogs continued from page 16)

polyps. In a follow up study the team at North Carolina State University developed a rapid and highly sensitive test to detect the presence of this mutation in cells shed into the urine (11). Importantly, the new test is not affected by the presence of bacteria or blood in the urine and provides a highly effective means to detect the presence of malignant TCC/UC cells. Dr. Breen's lab at NCSU has been randomly screening urine specimens from dogs over age 6 years, from breeds considered at high risk of developing a TCC/UC, including Westies.

The research team has already identified the presence of a suspected TCC/UC before the dog had any clinical signs of the disease. Subsequent examination of these dogs by their veterinarian, followed by high-resolution ultrasound, identified a very small mass. In these cases, dogs that scored positive for the presence of a BRAF mutation in their urine subsequently progressed to develop clinical signs over the following months. This is a very exciting step forward for the earliest detection and treatment of dogs with TCC/UC.

CADETSM BRAF Mutation Detection Assay For Early, Fast, And Reliable Detection Of Canine TCC/UC In Free-Catch Urine.

Sentinel Biomedical Inc. was founded by Dr. Breen's team at NCSU to allow this test to be made widely available across the nation. The team is developing a series of rapid tests to provide dog owners and veterinarians with access to reliable early CAnCER DETection in pet dogs. The CADETSM BRAF Mutation Detection Assay is the first early detection system for TCC/UC.

The CADETSM BRAF Mutation Detection Assay was designed specifically to identify tumor cells carrying the BRAF mutation, which is present in 85% of all TCC/UC cases. The test can detect as few as just 10 mutant bearing cells in a urine sample and has been detecting cases several months before any clinical signs associated with the cancer become evident. This enables owners of dogs that test positive to follow up with their veterinarian and seek the most appropriate treatment very early in the course of the disease, which is expected to improve both the quality and duration of the dogs' lives. Unlike previous and less discriminatory tests for canine TCC/UC, the CADETSM assay is unaffected by the presence of blood, protein, or bacteria in the urine.

Importantly, in all cases that have subsequently 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 TCC/UC in the biopsy of the mass. In contrast, this test does not have false positives; in studies of hundreds of controls, a BRAF mutation

has *NOT* been detected in specimens from dogs that were shown to not have the cancer.

The goal of screening for early detection of a TCC/UC is to allow earlier confirmation of diagnosis and thus provide more time to treat the cancer and not just the symptoms.

How Will This New Early Detection Of TCC/UC Help The Westie?

The West Highland White Terrier is a breed with an elevated risk of developing a TCC/UC. Studies have suggested that the Westie is 3-6x more likely to develop this cancer than the general dog population. The mean age of diagnosis of TCC in the Westie is considered to be 11 years and 95% of all cases are diagnosed in dogs age 6 years and older. Approximately 1 in 20 Westies with TCC/UC develop the cancer when under age 5 years. As with all dogs affected by TCC/UC, the majority of tumors in the Westie are detected in the bladder. The ability to reliably detect the presence of a TCC/UC earlier in the course of disease provides more time for the dog to be treated for this cancer.

Starting at 6 years of age, we recommend that urine samples be periodically collected from Westies and submitted for BRAF mutation detection. Our initial data suggest that this should be done every four months beyond age 6 years. Sentinel Biomedical have teamed with the American Kennel Club to offer an annual subscription for the CADETSM BRAF Mutation Detection Assay. Purchase of a CADETSM BRAF Mutation Detection Assay annual subscription provides all items needed to collect and submit three urine samples from one dog to be tested over the course of a year. Owners collect urine from their dogs and ship to the Sentinel Biomedical laboratory once every four months. Results are sent back within two weeks and if the BRAF mutation is detected the owner is advised to schedule an appointment with their veterinarian as soon as possible for follow-up.

In addition to screening for pre-clinical detection, Sentinel Biomedical also offer the CADETSM BRAF Mutation Diagnosis/Monitoring Assay. This version of the assay is used by veterinarians to help identify a TCC/UC in dogs that have developed symptoms suggestive of a possible TCC/UC. Results of this test are returned to the veterinarian within 2-3 days of the sample arriving at the testing laboratory. This product is available to veterinary professionals, and to dog owners under the guidance of their veterinarian.

The CADETSM BRAF kits are very easy to use. Owners collect free catch urine from their dog(s) at home in a clean household container and pour it into one of the collection jars provided. The jar is then shipped back to the lab to be tested. All packing materials and prepaid FedEx shipping labels are included with each kit.

(Continued on page 18)

Earlier detection will give you and your veterinarian more time to treat the cancer rather than treating the initial symptoms that emerge with the disease.

Be Part Of Nationwide Study Of Canine TCC/UC

We share the same kinds of spontaneous cancers and environmental exposures with our dogs. With highly sophisticated analytical tools now available, our dogs provide scientists with an ideal population for genetic research. Early detection tests being developed and offered by Sentinel Biomedical's CADETSM program not only provide opportunities to potentially extend lifespans of beloved pets, but also offers researchers valuable insight that can be applied to benefit human cancer patients.

Along with each submission dog owners are offered the opportunity to be part of a large nationwide research study to investigate the genetic and environmental factors associated with TCC/UC of the bladder and prostate. This study will provide valuable data to help your breed and other breeds diagnosed with these cancers, and all participants will have access to updates of the research program to learn how their dogs has contributed.

Our dogs truly are our best friends, in the home and in the fight against cancer.



For further information about the CADETSM BRAF Mutation Detection Assay please e-mail CADETBRAF@SentinelBiomedical.com

Further Reading – The Science Behind The Testing.

- 1) 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. PubMed PMID: 26053201; PubMed Central PMCID: PMC4460039.
Review study at: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0129534>
This study reports on the presence of the *BRAF* mutation across numerous canine cancers, highlighting the high frequency of the mutation in canine TCC/UC.
- 2) 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.
Review study at: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0144170>
This study reports on the development of the *BRAF* mutation assay used to detect the presence of the mutation in urine specimens.
- 3) 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.

Review study at: <http://mcr.aacrjournals.org/content/13/6/993.long>

This study reports on an independent discovery of the *BRAF* mutation in canine TCC.

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8. Nyland TG, Wallack ST, Wisner ER. Needle-tract implantation following us-guided fine-needle aspiration biopsy of transitional cell carcinoma of the bladder, urethra, and prostate. Veterinary radiology & ultrasound : the official journal of the American College of Veterinary Radiology and the International Veterinary Radiology Association. 2002;43(1):50-3. Epub 2002/02/28. PubMed PMID: 11866046.
9. 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. PubMed PMID: 26053201; PubMed Central PMCID: PMC4460039.
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11. 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. doi: 10.1371/journal.pone.0144170. PubMed PMID: 26649430.