YEAR IN REVIEW: CANCER DIAGNOSIS AND TREATMENT IN 2023

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It continues to be an exciting time in the world of veterinary oncology with new treatments and diagnostics to help not only diagnose cancers earlier but offer novel therapies to owners.

1. **STELFONTA® (tigilanol tiglate injection) 1 mg/mL INTRODUCTION**

STELFONTA® (tigilanol tiglate injection) is approved by the FDA as a prescription intratumoral injection indicated for the treatment of nonmetastatic cutaneous mast cell tumors and nonmetastatic subcutaneous mast cell tumors located at or distal to the elbow or the hock.[3] Tigilanol tiglate is part of a novel small molecule class of drugs called epoxy-tiglianes. It is isolated from the seed of *Fontainea picrosperma* (blushwood tree). It is its unique mode of action that sets it apart from other local treatment therapies. This is being covered in a separate talk at ISVMA.

2. **THE NU.Q® VET CANCER SCREENING TEST**

Liquid biopsy is an emerging field in human medicine with significant potential in veterinary medicine. It enables the use of non-invasive techniques to analyze tumor-derived material, including circulating tumor cells, extracellular vesicles, and cell-free DNA. Compared to traditional tissue biopsies or expensive imaging tests, liquid biopsy offers numerous advantages. Information provided through these tools in cancer patients can provide early detection of neoplastic disease, provide prognostic information, monitor response to treatment, and help identify druggable targets.[1,2] Furthermore, liquid biopsy assays are much more amenable to serial testing when compared to traditional tissue biopsies or expensive imaging tests. In this lecture, we will explore the applications and benefits of liquid biopsy in veterinary oncology.

Understanding the Science Behind Liquid Biopsy:

To understand liquid biopsy, we need to delve into its scientific foundations. Within a cell's nucleus, DNA is compacted into nucleosomes, which are bead-like structures comprised of DNA coiling around histone proteins. In the case of human or canine cancer, nucleosomes from cancer cells are released into the bloodstream. By utilizing antibodies specific to nucleosomes, the Nu.Q® Vet Cancer Test can measure and analyze these nucleosomes. The Nu.Q® Vet Cancer Test utilizes this approach to identify potential cancer cases in veterinary patients[3]. This must be confirmed by follow up procedures to confirm the suspicion of cancer – for example, an aspirate, biopsy, or imaging. Early diagnosis and monitoring are crucial aspects of cancer care, and liquid biopsy has the potential to significantly improve treatment outcomes and enhance the quality of life for dogs. The Nu.Q® Vet Cancer Screening not only aids in the detection of cancer but also provides valuable additional information to inform the clinical decision-making process, empowering veterinary professionals to make informed and impactful choices for their patients. The Nu.Q® Vet Cancer Test offers the advantage of convenient and cost-effective serial testing compared to traditional methods. This accessibility facilitates regular monitoring of patients' cancer status throughout their treatment journey.

The Nu.Q® Vet Cancer Test as a Screening Test

The Nu.Q® Vet Cancer Test is a simple, affordable, easy to use screening blood test for all dogs (7 years and older) and younger dogs (4 years and older) with an increased risk of developing cancer in their lifetimes, due to breed disposition or family history, including: Labrador Retriever, French Bulldog, Golden Retriever, German Shepherd, Beagle, Rottweiler, Boxer, Pembroke Welsh Corgi, Great Dane, Miniature Schnauzer, Siberian Husky, Bernese Mountain Dog, Mastiff, Irish Wolfhound, Flat Coated Retriever, and Scottish Wolfhound. .

In a peer-reviewed and published case series of 662 dogs, the Nu.Q® Vet Cancer Test was shown to detect 76% of systemic cancers; lymphoma (77%), hemangiosarcoma (82%), and histiocytic sarcoma (54%), and was able to identify approximately 50% of all cancers researched at 97% specificity[4]. Lymphoma is the most common form of canine cancer and together with hemangiosarcoma make up approximately one-third of all cancers.

The Nu.Q® Vet Cancer Test as a Monitoring Test for Canine Lymphoma

Clinicians commonly use existing tools such as physical exam findings, blood work, lymph node aspirates, radiographs, and ultrasound to monitor lymphoma patients for treatment response and remission. To date, there has been a lack of useful circulating biomarkers available to veterinary oncology patients. In a recent study including 37 dogs with lymphoma, circulating plasma nucleosome concentrations were evaluated at diagnosis, throughout treatment, and during remission monitoring. Additionally, C-reactive protein and thymidine kinase-1 levels were recorded for comparison. Plasma nucleosome concentrations were significantly higher at diagnosis and progressive disease than they were when dogs were in remission. All but two dogs had plasma nucleosome concentrations that returned to the low range during treatment. These two dogs had the shortest progression free and overall survival times. Dogs with the highest plasma nucleosome concentrations had a significantly shorter first progression free survival than dogs with lower plasma nucleosome concentrations at diagnosis. Plasma nucleosome concentrations correlated better with disease response and progression than either thymidine kinase or C-reactive protein.

Key Findings

- Nucleosome levels were consistently low across normal healthy control dogs.
- Nucleosome levels were elevated in lymphoma and hemangiosarcoma and variable across patients.
- The top 4 malignancies detected by the test included lymphoma, hemangiosarcoma, histiocytic sarcoma, and malignant melanoma.
- At a specificity of 97%, 50% of all cancers studied were detected.
- The Nu.Q® Vet Cancer Screening test detects a variety of lymphoma stages and phenotypes.
- The Nu.Q® Vet Cancer Screening test also has high specificity and sensitivity in detecting all stages of hemangiosarcoma.
- Note: elevated levels have been observed in a variety of infectious and inflammatory diseases and are not specific for a particular cancer type. Results should be interpreted in clinical context in combination with history, physical exam, and other diagnostic methods.
- Localized tumors such as soft tissue sarcomas are less likely to cause elevations in plasma nucleosomes.
- Further research examining the use of the test for disease progression and treatment monitoring in dogs diagnosed with B-cell lymphoma is currently under peer-review for future publication.

To submit a sample:

- Patients should be fasted (minimum four hours) for this test to be accurate.
- Draw down 2-5 mL of blood from a peripheral vein.
- Immediately fill EDTA tube (purple top) with blood.
- Spin the sample in-house at 1600xg (the blood spin) for 10 minutes within one hour of sampling.
- Remove plasma and place in a non-additive tube (red top). Be careful to not disturb buffy coat.
- Ship sample with cold packs. Store in fridge until ready to ship*
    - *Please refer to the preferred reference lab for specific sample shipping logistics.

The Nu.Q® Vet Cancer Test identifies patients who may have cancer, however, not all neoplastic conditions are detectable using elevated plasma nucleosomes. Localized tumors are less likely to cause elevated plasma nucleosomes, and this test is not able to differentiate severe/systemic inflammation from cancer. The benefit

for the veterinarian, the owner, and the dog is a streamlined diagnostic process: simpler and quicker diagnosis with the goal of providing quality of life to the pet and more quality time with its owners, as well as providing valuable additional information to inform the clinical decision-making process.

Where to find the Nu.Q® Vet Cancer Test:

- **Idexx**
  - IDEXX Nu.Q® Canine Cancer Screen
  - Test Code – 8993
- **Heska**
  - Heska Nu.Q® Canine Cancer Screen and Monitor
  - Reference Lab Test Code – 313100
  - Point of Care Test – See Heska booth or representative for more details
- **Texas A&M University GI Lab**
  - Nu.Q® Vet Cancer Test
  - Test available via GI Lab Clinic portal
- **Outside the US:**
  - Portugal: DNATech
  - Italy: Scil (Heska)
  - Coming soon: Canada, UK, Japan

3. LAVERDIA-CA1 FOR CANINE LYMPHOMA

In January 2021, the US FDA conditionally approved Laverdia-CA1 (verdinexor) to treat dogs with lymphoma. It is licensed to Anivive Lifesciences Inc. Laverdia-CA1 (verdinexor) is a novel orally bioavailable selective inhibitor of nuclear export (SINE) that exhibited anti-tumor activity against non-Hodgkin lymphoma in a prior phase I study. Laverdia-CA1 works to prevent certain proteins from leaving the nucleus of cancer cells, thereby allowing these proteins to control the growth and prevent the spread of cancerous cells in dogs. Laverdia-CA1 is given orally twice per week, with at least 72 hours between doses.

The reasonable expectation of effectiveness of Laverdia-CA1 was established in a study with 58 client-owned dogs with B- or T-cell lymphoma who were followed for at least eight months. The dogs were either newly diagnosed with lymphoma (naïve) or were in their first relapse after completing a single or multi-agent chemotherapy regimen. The study included dogs of varying breeds, weights, and both genders, with the majority of the dogs having lymphoma stage III (generalized lymph node enlargement). Seventeen of the 58 dogs (29%) did not show progression of lymphoma for at least 56 days after taking verdinexor. Three of these dogs did not show any progression for at least 182 days. Treatment with single-agent, orally administered Laverdia resulted in an objective response rate (ORR) of 37%, of which dogs with T-cell lymphoma had an ORR of 71%. The median TTP was 43 days for naïve lymphoma patients (n = 33). The median TTP for relapse lymphoma patients was 24 days (n = 21). Forty percent of patients remained on the study for at least 8 weeks, suggesting a substantial proportion of canine lymphoma patients have a significant, sustained benefit from the treatment.

Laverdia was well tolerated in all dose groups with grade 1-2 anorexia being the most common adverse event. Anorexia was responsive to symptomatic and supportive medications, including prednisone. The other most common adverse reactions were vomiting, diarrhea, weight loss, lethargy, polyuria, polydipsia, elevated liver enzymes, and thrombocytopenia. Laverdia-CA1 should be given to dogs immediately after eating, as this increases the amount of drug absorbed into the bloodstream.
The package insert for prescribing veterinarians includes detailed user safety information and special instructions for handling and administering the drug. Gloves tested for use with chemotherapy drugs should always be worn when handling Laverdia-CA1 and cleaning up after a dog undergoing treatment and for three

days following the last treatment. This includes handling the dog's food and water bowls, as well as feces, urine, vomit, or saliva from the dog. Laverdia-CA1 also comes with a client information sheet for prescribing veterinarians to give to their clients. This sheet is written specifically for dog owners and explains how to safely handle Laverdia-CA1, how to safely clean up after a dog undergoing treatment and other important safety information.

4. CANALEVIA

Canalevia-CA1 is a FDA conditionally-approved product to treat chemotherapy-induced diarrhea (CID) in dogs. Canalevia-CA1 has a novel mechanism of action that normalizes the fluid influx into the intestinal lumen. The active ingredient crofelemer acts within the lumen of the GI tract, targeting channels on the luminal membrane of epithelial cells lining the intestine.

Canalevia-CA1 treats CID by modulating the hypersecretion of Cl^- in diarrhea and normalizes fluid influx into the intestinal lumen. Secretory diarrhea (intestinal fluid secretion) is driven by active transepithelial Cl^- secretion, which creates the electrochemical force for paracellular Na+ secretion and the osmotic driving force for transcellular fluid secretion. Crofelemer in Canalevia-CA1 normalizes the hypersecretion of both the cyclic adenosine monophosphate (cAMP)-stimulated cystic fibrosis transmembrane conductance regulator (CFTR) chloride (Cl) channel and the calcium-activated Cl channel (CaCC) at the luminal membrane of intestinal enterocytes. Dysregulation of the CFTR and CaCC channels increases the osmotic gradient and causes excessive fluid influx into the lumen, resulting in secretory diarrhea.

Canalevia-CA1 is a delayed-release tablet product that contains the active ingredient crofelemer, a plant-based botanical product for the treatment of CID in dogs. Derived from the latex of the *Croton lechleri* tree, crofelemer is a first-in-class antidiarrheal agent with a unique physiological mechanism of action for chloride ion channel regulation. Canalevia-CA1 acts locally, and it is not absorbed into the bloodstream, leading to a well-tolerated and non-toxic drug product.

The dose is a 125 mg tablet orally twice daily for 3 days for dogs weighing up to 140 pounds. For dogs weighing more than 140 pounds, administer two tablets orally twice daily for 3 days. Tablets should be swallowed whole and should not be broken, crushed, or chewed. If the dose is chewed, one additional dose may be administered.

In addition to being FDA conditionally approved for canine CID, advantages include it is not an antibiotic, it is a natural and plant-based product, it has a low risk of constipation and it normalizes fluid influx into the intestinal lumen.

5. THE CADET BRAF/PLUS TEST FOR CANINE UROTHELIAL CARCINOMAS

Urothelial carcinomas (UC) include tumors of the lower urinary tract and prostatic carcinoma (PC). These are aggressive genitourinary cancers in dogs, characterized by invasion to surrounding tissues and high metastatic potential. Urothelial carcinoma affects the bladder, urethra and kidneys of male and female dogs and also the prostate of males. Diagnosis is often based upon signalment, ultrasound, traumatic catheterization, and urine cytology, but definitive diagnosis is limited to histopathology via cystoscopy or surgical exploratory, requiring an invasive and costly biopsy. There has been a need for a non-invasive test for UC.

The CADET® ***BRAF*** **Assay** can be used for diagnosis and monitoring of UC including canine transitional cell carcinoma (TCC) and prostatic carcinoma (PC). This test offers a less invasive way to diagnose these cancers. Recent studies identified a mutation (V595E) in the canine *b-raf* gene in a large proportion of canine urothelial carcinoma (UC), which include TCC and prostatic carcinoma (PC). In assessing various cancers including epithelial, mesenchymal, and hematopoietic, the V595E mutation was identified in canine UC with the highest

penetrance rates of up to 87%. Knowing bladder and prostatic cancers shed tumor cells into urine, the presence of the V595E mutation in urine appeared to be an excellent molecular diagnostic marker. The polymerase chain reaction (PCR) assay of urine tests for a common mutant gene in UC cancers. The test is 85% sensitive and 100% specific for canine urothelial carcinoma and prostate carcinoma patients. (Mochizuki)

The assay has since been validated in hundreds of clinical cases, demonstrating the mutation is not present in the urine of healthy dogs, or from dogs that have benign bladder diseases (bladder polyps, inflammation, or chronic cystitis). In cases in which a biopsy of a mass was performed, there was concordance between BRAF mutation-positive in free-catch urine and pathology-based confirmation of a bladder/prostatic carcinoma. As such, the presence of the mutation in canine urine is therefore a highly specific indicator of the presence of a UC. In addition, the CADET® BRAF-PLUS assay was launched commercially in July 2018 and will be run on every BRAF mutation undetected case automatically. This assay detects a copy number variation in 2 out of 3 non-b-raf mutations TCC/UC. The new test increases overall sensitivity to detect a TCC/UC from 87% to >95%. This translates to only 5% false negatives. The Cadet BRAF test is exclusively available through Antech Diagnostics.

Potential advantages of the assay:

- The free-catch urine system is fully non-invasive. Urine samples can be collected while the patient is at the clinic or by the owner at home.
- The assay permits detection for earliest detection of emerging UC, including TCC – even before overt signs of the cancer become evident.
- With the add-on Cadet BRAF Plus test, there are only 5% false negatives
- 0% false positives
- Timely detection of UC allows owners to direct their resources toward effective treatment of the cancer itself, rather than the non-specific clinical signs.
- Unlike prior bladder cancer tests, the test is not affected by the presence of blood, protein, glucose, or bacteria in the urine.

How can we use the test in practice knowing early detection & diagnosis are important for UC?

- I am currently using the **CADET®** *BRAF* test as a **diagnostic** for UC. A positive BRAF testing can substitute for tissue diagnosis (histology, cytology). If the *BRAF* mutation is not detected, the urine specimen is then assessed by the lab using a proprietary algorithm to determine whether it meets the criteria for analysis with **CADET®** *BRAF*-**PLUS.**
- I am also using serial **CADET®** *BRAF/PLUS* tests to **monitor** during chemotherapy to assess response to treatment, often in conjunction with bladder ultrasound. This provides an objective response to see how an individual patient's tumor is responding to chemotherapy.
- Educate our clients about high risk breeds. But remember other breeds & mixed breeds can sadly be diagnosed too. If a client has a high-risk breed, consider screening with the **CADET®** *BRAF* test. I consider **screening** at-risk breeds every 4 to 6 months with this easy-to-collect urine test.
- If a patient, especially over the age of 6, has lower urinary tract clinical signs or multiple UTI, I recommend additional tests (abdominal ultrasound, BRAF urine test, cytology or biopsy) - it will depend on the case. Consider referral to a specialist.

Clinical signs for UC are often subtle, nonspecific and confused for other lower urinary tract diseases. The **CADET®** *BRAF* test provides a non-invasive, easy-to-collect urine test for earliest detection of emerging UC – even before overt signs of the cancer become evident. Timely detection of UC allows owners to direct their resources toward effective treatment of the cancer itself, rather than the non-specific clinical signs. If your patient is diagnosed with TCC or PC, there are treatment options. The most common ones are chemotherapy, NSAIDs, and

sometimes surgery but there are others, so consider referral or consultation with an oncologist. Treatment is well tolerated, and treated dogs typically live longer than untreated dogs.

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