WHAT'S NEW IN CANCER DIAGNOSIS AND THERAPY IN 2024

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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.

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 the MCT talk.

GILVETMAB

Gilvetmab is the first conditionally licensed caninized anti-PD-1 monoclonal antibody in veterinary medicine. It is labeled for use in dogs with stage I, II, III MCT and stage II or III melanoma. Gilvetmab targets the programmed cell death protein 1 (PD-1) pathway in dogs and blocks interaction of PD-1 receptor on activated T cell with its ligand PD-L1 or PD-L2. These PD-L1 or PD-L2 ligands can be overexpressed on some tumor cells and supporting cells in the tumor microenvironment. This overexpression is a tactic by cancer cells to evade immune detection and destruction. The inhibition of interaction prevents suppression of T-cells allowing it to go on to recognize and facilitate killing of tumor cells. This therapeutic approach is designed to boost the immune system's response against cancer cells. By inhibiting this interaction, gilvetmab essentially "unmasks" the cancer cells, allowing the dog's immune system to recognize and attack them.

Setting realistic expectations for checkpoint inhibitor monotherapy is essential. A review by Mao et al. analyzed the objective response rate (ORR) for anti-PD-1 or anti-PD-L1 monotherapy across 31 human cancers. Monotherapy results showed the highest ORR peaking at just below 40%, with the majority ranging from 15% to 25%. Notably, an enhanced ORR is observed when predictive biomarkers are utilized, or when combined with other therapies, although such advanced diagnostics may not be widely available in veterinary medicine for some time. In human oncology, augmented antitumor efficacy has been documented with the adjunctive use of therapies such as radiation, chemotherapy, angiogenesis inhibitors, targeted therapy, additional immune checkpoints inhibitors, fecal microbiota transplantation, epigenetic modulators, and metabolic modulators. Moreover, some studies have indicated that patients who had antibiotic treatments 2 to 6 weeks prior to starting immune checkpoint inhibitors (ICIs) experienced reduced progression-free survival (PFS) and overall survival (OS).

Research and clinical trials focusing on gilvetmab's efficacy in veterinary oncology are underway to determine its impact on survival rates, tumor shrinkage, and the potential for complete remission in dogs with cancer. In a pivotal clinical study encompassing 26 dogs with stage I, II, or III MCT, the ORR for the target lesion reached 73%, with cases showing either a reduction in size or stable disease. The complete response (CR) was recorded at 8%, partial response (PR) at 38%, and stable disease (SD) at 27%. Among the 24 dogs suffering from stage II or III melanoma, the ORR was 60%, with a CR of 8%, a PR of 12%, and an SD of 40%.

The administration of gilvetmab involves a 30-minute intravenous infusion at a dose of 10 mg/kg every two weeks, capped at a maximum of 10 treatments. It is advised to premedicate with diphenhydramine intramuscularly to minimize the risk of infusion-related allergic reactions. Additionally, patients should be observed for one hour post-infusion to promptly address any immediate side effects. The decision to extend treatment beyond 10 cycles rests with the treating veterinary oncologist and may be considered necessary in certain cases.

Side effects, while critical to consider with any new treatment, are generally manageable in the case of gilvetmab. The majority of adverse events in dogs are categorized as grade 1 or 2. Typical side effects include lethargy, reduced appetite, gastrointestinal discomfort, and in rarer instances, more severe immune-mediated conditions. Veterinarians must diligently monitor and address any adverse reactions to ensure optimal outcomes for their canine patients.

In summation, gilvetmab's advent into veterinary oncology heralds a new era in the management of canine cancers, offering a novel approach to stimulating an immune-mediated assault on cancer cells. The ongoing accumulation of research and clinical data will progressively elucidate the specific role, effectiveness, and safety of gilvetmab in canine cancer therapy.

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 a 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 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
- **Outside the US:**
    - Portugal: DNATech
    - Italy: Scil (Heska)
    - Coming soon: Canada, UK, Japan

LAVERDIA-CA1
In January 2021, the US FDA conditionally approved Laverdia-CA1 (verdinexor) to treat dogs with lymphoma and 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.

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. 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 blood stream, leading to a well-tolerated and non-toxic drug product. The dose is 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 normalized fluid influx into for the intestinal lumen.

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