Cancer detection in 2022: Can a blood test really find 30 different types of cancer in dogs?

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Disclosures:

Dr. Andi Flory is the Chief Medical Officer and co-founder of PetDx.

Earlier this year, the CANcer Detection in Dogs (CANDiD) study was published, detailing the clinical validation of a novel "liquid biopsy" test for the detection of 30 different cancer types in dogs from a simple blood draw. Data from this landmark study, which involved over 1,000 cancer-diagnosed and presumably cancer-free dogs, will be presented. The session will review the clinical applications of this innovative cell-free DNA-based technology and provide initial laboratory experience showing how this testing is being used in veterinary practices across the country. The session will close with a series of interesting case studies demonstrating the real-world applications of liquid biopsy testing as a cancer screening tool for dogs at higher risk of cancer, and as an aid-in-diagnosis for dogs in which cancer is suspected.

Speaker Bios:

Dr. Andi Flory, DVM, DACVIM (Oncology)

Chief Medical Officer

Dr. Flory is a specialist in medical oncology with nearly two decades of experience practicing and publishing in the areas of early cancer diagnosis, treatments, trials, and novel diagnostic test evaluation. A diplomate of the American College of Veterinary Internal Medicine in oncology, Dr. Flory graduated from the Ohio State University of College of Veterinary

Medicine and completed additional training at Florida Veterinary Specialists and Cancer Treatment Center in Tampa, Florida, and Cornell University.

In 2019, she treated a small dog named Poppy for pancreatic cancer. Poppy lost her battle with the disease, but left an indelible mark on Dr. Flory, leading her to a newfound passion for cancer genomics. Soon after, Dr. Grosu tapped Dr. Flory to launch PetDx and serve as its first chief medical officer.

Prior to PetDx, most recently she co-directed the oncology internship and served as a medical oncologist at Veterinary Specialty Hospital in San Diego. Dr. Flory founded the medical oncology service at a sister hospital, Veterinary Specialty Hospital - North County. Prior to that she was a staff oncologist, resident advisor and co-chief of the oncology department at The Animal Medical Center in New York City. She has served as a principal investigator for national and international multi-site clinical investigational studies.

Dr. Flory is constantly expanding her knowledge in cutting-edge areas of veterinary medicine. She holds a certificate in genomics from The Johns Hopkins University along with certificates in cancer genomics and precision oncology, and genetic testing and sequencing technologies from Harvard Medical School. In addition, Dr. Flory is a skilled lecturer. An avid snowboarder and passionate foodie, when not keeping up with two preschoolers, Dr. Flory loves to travel, spend time with her husband, sons, their cat Mochi, and dog Cheyenne.

Learning Objectives:

1. Define "liquid biopsy" and examine how next-generation sequencing can be used to identify cancer-associated genomic alterations in the blood of dogs.
2. Examine initial laboratory experience, including: ordering trends, test results in submitted cases, and available outcome data.
3. Identify real-world opportunities for liquid biopsy incorporation into routine preventive care exams or as an aid-in-diagnosis when cancer is on the list of differential diagnoses.

Agenda:

- Background and clinical validation
- Study details and results
- Clinical applications
    - Examples of uses
    - Laboratory information
- Case Studies

Clinical Validation of OncoK9®, a Blood-Based Multi-Cancer Early Detection "Liquid Biopsy" Test for Dogs

KEY TAKEAWAYS

1. An international, multi-site clinical validation study in a population of 1,100 dogs, including over 40 distinct cancer types.
2. The blood-based liquid biopsy test had high sensitivity and a very low 1.5% false positive rate, demonstrating performance comparable to similar human liquid biopsy tests.
3. Successfully detected 30 different cancer types from a simple blood draw with the liquid biopsy test validated in the CANDiD study.

INTRODUCTION

Cancer is the leading cause of death in dogs,[1] yet there are no established screening paradigms for early detection. As a result, many patients are diagnosed at an advanced stage, when clinical signs have developed, the ability to provide long-term control is low, and prognosis is poor. Liquid biopsy methods that interrogate cancer-derived genomic alterations in cell-free DNA (cfDNA) fragments in blood[2] are being adopted for early cancer detection in human medicine[3] and are now clinically available for use in veterinary medicine.[4] The recently published CANcer Detection in Dogs (CANDiD) study, the largest clinical validation study ever performed in veterinary cancer diagnostics, is an international, multi-center clinical study designed to validate the performance of OncoK9®, a novel, multi-cancer early detection (MCED) "liquid biopsy" test using next-generation sequencing (NGS) of blood-derived DNA, specifically developed for the non-invasive detection and characterization of cancer in dogs.[5]

The time it takes for cancer to progress is well established for many cancer types in humans (colorectal, pancreatic, etc.), and this known latency period – ranging from many years to decades – is used to inform recommendations for the appropriate screening interval for each cancer. In dogs, cancer progression is less well studied, but latency periods of at least 1-3 years (and in some cases 10+ years) can be reasonably estimated based on existing literature.[6] The goal of a cancer screening program is to detect cancer in a preclinical state, before the onset of clinical signs;[7] early detection and treatment are the best ways to manage cancer in pets, as cancer is frequently treatable and earlier diagnosis will aid veterinarians in delivering the best care possible.[8,9] Considering the estimated timeframe for cancer development and the median age at which cancer is typically diagnosed in dogs, a prudent recommendation would be to start annual screening no later than 7 years of age for all dogs; and as early as 4 years of age for dogs belonging to breeds with an earlier median age at cancer diagnosis (Saint Bernard, Mastiff, Great Dane, Bulldog, etc.).[6]

INTENDED USE

OncoK9 is a multi-cancer early detection test for the detection and characterization of cancer-associated genomic alterations in DNA isolated from canine whole blood samples, using next-generation sequencing (NGS) technology. OncoK9 is intended for use in dogs who are at higher risk of cancer. It is recommended as an annual screening test for dogs at higher risk of cancer due to age and/or breed, as described above; and as an aid-in-diagnosis for dogs in which cancer is suspected based on clinical signs or other clinical findings. As with any laboratory test, OncoK9 results should be interpreted by a veterinarian in the context of each patient's medical history and clinical presentation. This test is available by prescription only.

CLINICAL VALIDATION OF ONCOK9

Prior to clinical validation, analytical performance of the test was assessed using contrived samples, generated by mixing DNA from well-characterized canine cancer cell lines into DNA extracted from the white blood cells of a single cancer-free canine subject at defined genomic equivalent ratios. Repeatability was assessed by analyzing agreement among multiple within-run replicates,

TABLE 1. Demographic and clinical characteristics of subjects in the testing set of the CANDiD study

DEMOGRAPHIC & CLINICAL CHARACTERISTICS	DISPOSITION OF SUBJECTS (n=876)
Breed	Purebred: 434 subjects Mixed-breed: 442 subjects
Sex	Male: 463 Female: 413
Age	Median: 6.6 years Range: 1.0 - 15.8 years
Weight	Median: 29.1 kg Range: 6.0 - 106.8 kg
Cancer Status	Cancer-diagnosed: 352* subjects Presumably cancer-free: 524* subjects

**One of the cancer-diagnosed subjects and three of the presumably cancer-free subjects received Indeterminate test results and were excluded from analysis of test performance (leaving 351 cancer-diagnosed and 521 presumably cancer-free subjects for analysis).*

processed by the same operators under the same conditions. Reproducibility was assessed by analyzing agreement among replicates across multiple runs, operators, days, and reagent lots. The repeatability and reproducibility were each >95%.

Clinical validation of OncoK9 in the CANDiD study included a total of 1,100 prospectively collected whole blood samples from client-owned dogs, with and without cancer, at 41 clinical sites across the US, Canada, Brazil, the Netherlands, France, and Hong Kong between November 2019 and August 2021.[a] Collection sites included private veterinary specialty practices, university/academic veterinary hospitals, and general practices.

The 1,100 subjects included in the validation of the test met study enrollment and laboratory QC criteria, and for each subject, a valid test result was generated. Subjects were randomly assigned to independent training (n=224) and testing (n=876) sets. Bioinformatics algorithms were optimized using the training set, and the locked-down pipeline was subsequently applied to the testing set to establish the test's clinical performance characteristics. All data reviewers were blinded to the cancer status and type of cancer in patients until after test results were issued.

The testing set, from which test performance was derived, comprised 352 cancer-diagnosed dogs (subjects with a definitive diagnosis of a malignant tumor) and 524 presumably cancer-free dogs (subjects presumed to be cancer-free due to no history of cancer and no suspicion of cancer based on a thorough history and physical exam by the treating veterinarian at the time of study enrollment). The cancer-diagnosed subjects in the testing set represented the full spectrum of cancer stages, with 205 (58%) localized/regional[b] cases, 136 (39%) disseminated/metastatic[c] cases, and 11 (3%) cases where the extent of disease was undetermined.[d] **Table 1** summarizes the demographic and clinical characteristics of all subjects in the testing set.

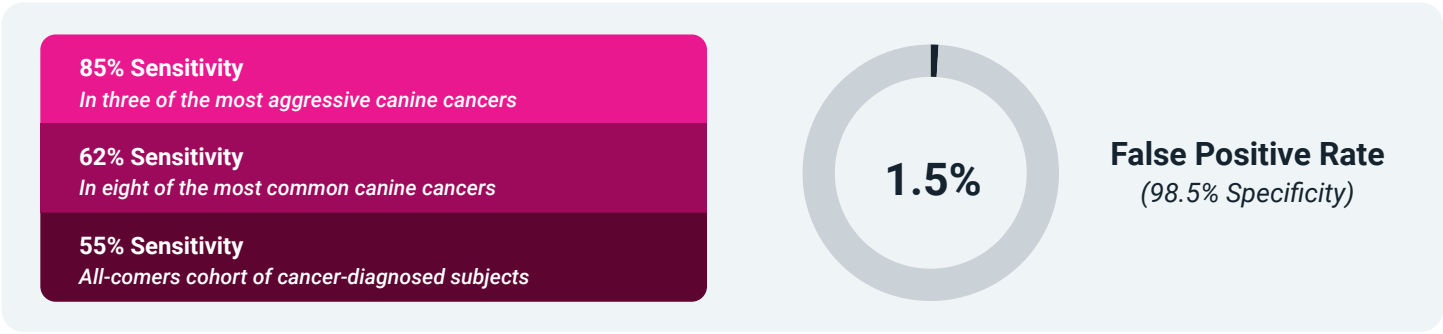
ONCOK9 PERFORMANCE IN THREE OF THE MOST AGGRESSIVE CANINE CANCERS

Lymphoma,[e] hemangiosarcoma,[f] and osteosarcoma are highly aggressive canine cancers. In the testing set, there were 137 total subjects with a single primary cancer belonging to one of these three cancer types. The liquid biopsy test returned a *Cancer Signal Detected* (positive) result in 117 of these 137 subjects, resulting in an overall detection rate of 85.4% (95% CI: 78.4 – 90.9%) across these three aggressive cancer types. **(Figure 1)**

ONCOK9 PERFORMANCE IN EIGHT OF THE MOST COMMON CANINE CANCERS

Certain types of cancer are more common in dogs and account for the majority of cancer mortality in the species, notably these eight: lymphoma,[e] hemangiosarcoma,[f] osteosarcoma, soft tissue sarcoma, mast cell tumor, mammary gland carcinoma, anal sac adenocarcinoma, and malignant melanoma.[10] In the testing set, there were 236 subjects with a single primary cancer belonging to one

FIGURE 1. OncoK9 performance rates in pre-specified cohorts



of these eight cancer types that received a positive or negative result. The liquid biopsy test returned a *Cancer Signal Detected* (positive) result in 146 of these 236 subjects, resulting in an overall detection rate of 61.9% (95% CI: 55.3 – 68.1%) across these eight common cancer types. **(Figure 1)**

ONCOK9 PERFORMANCE FOR MULTI-CANCER DETECTION

Overall, there were 351 cancer-diagnosed subjects in the testing set that received a positive or a negative result. In this cohort, the test returned a *Cancer Signal Detected* (positive) result for 192 subjects, for an overall sensitivity (detection rate) of 54.7% (95% CI: 49.3-60.0%).[g] OncoK9 detected cancer signal from 30 distinct cancer types in 433 cancer-diagnosed subjects evaluated across the training and testing sets.

There were 521 presumably cancer-free subjects in the testing set that received a positive or a negative result. In these subjects, the test returned a *Cancer Signal Not Detected* (negative) result for 511 dogs, and *Cancer Signal Detected* (positive) result for 10 dogs ("putative false positives", pFP). Two pFP subjects were diagnosed with cancer after undergoing a confirmatory cancer evaluation triggered by a *Cancer Signal Detected* result, and were excluded from analysis of test performance; these subjects were diagnosed with cancer 5 months (hemangiosarcoma and 19 months (lymphoma) following collection of the blood samples, respectively, and did not have clinical signs of cancer at the time of diagnosis. The remaining 519 subjects were used for the calculation of specificity. The overall specificity of the test was 98.5% (511/519; 95% CI: 97.0 – 99.3%), corresponding to a false positive rate (FPR) of 1.5% (95% CI: 0.7 – 3.0%).[h] **(Figure 1)**

FIGURE 2. OncoK9 detection rates in eight of the most common canine cancers

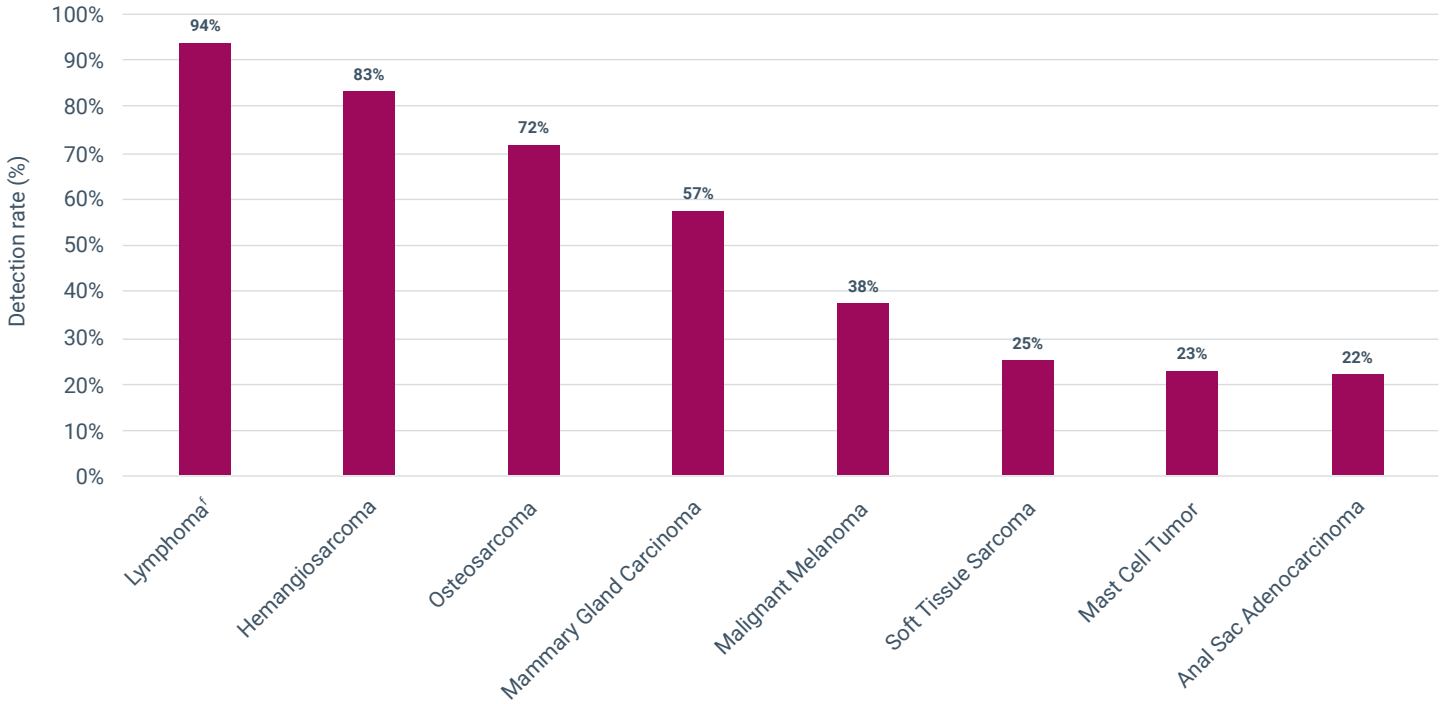


TABLE 2. Positive and negative predictive values based on estimates of prior probabilities of cancer in two intended use populations

Clinical use case	Intended use population	Prior probability of cancer*	PPV (Given a positive test result)	NPV (Given a negative test result)
Screening	≥ 7 years old and/or predisposed breed	8 - 10%	76 - 80%	95 - 96%
Aid-in-diagnosis	Cancer suspected based on clinical presentation	30 - 50%	94 - 97%	68 - 84%

**Estimated prior probabilities are based on a review of the literature and a survey of over 300 US based veterinarians (PetDx data on file).*

Estimated ranges for positive predictive value (PPV) and negative predictive value (NPV), calculated using a test sensitivity of 54.7% and specificity of 98.5% (ranges calculated using the lower and higher ends of prior probability).

Among the 8 common canine cancers in the training and testing sets, OncoK9 demonstrated higher sensitivity for cancers that tend to be more aggressive, such as lymphoma and hemangiosarcoma; while other cancers that may be easier to identify by physical examination early in the course of the disease had lower sensitivity, such as mast cell tumor and anal sac adenocarcinoma. **(Figure 2)**

POSITIVE AND NEGATIVE PREDICTIVE VALUES

At the current sensitivity and specificity, and using an estimated prevalence of cancer in the "screening" and "aid-in-diagnosis" intended use populations of 8-10% and 30-50% respectively, the positive predictive value (PPV) of OncoK9 can be determined. As shown in **Table 2**, when a positive (*Cancer Signal Detected*) result is issued, the PPV is expected to range from 76% (in the screening use case) to 97% (in the aid-in-diagnosis use case), meaning that a large majority of patients who receive a positive OncoK9 result will prove to be clinically positive for cancer. In the context of a negative (*Cancer Signal Not Detected*) OncoK9 result, the negative predictive value (NPV) is expected to range from 68% (in the aid-in-diagnosis use case) to 96% (in the screening use case); this underscores the importance of further evaluation if the liquid biopsy test result is negative but cancer remains high on the list of differential diagnoses.

CANCER SIGNAL ORIGIN PREDICTION

In subjects who received a *Cancer Signal Detected* result, and prior to unblinding, the sequencing data were reviewed using proprietary algorithms for genomic features specific to hematological malignancies. A total of 96 subjects with a diagnosis of hematological malignancy (lymphoma or lymphoid leukemia) received a *Cancer Signal Detected* result. Of these 96 subjects, 40% (n=38) were assigned a Cancer Signal Origin (CSO) prediction of "hematological malignancy (likely lymphoma or lymphoid leukemia)", while the remaining 58 did not receive a CSO prediction; three subjects who had been diagnosed with non-hematological malignancies were additionally assigned this CSO prediction, for an overall CSO prediction accuracy of 92.7% (38/41) for hematological malignancy.

EVALUATION OF PRE-ANALYTICAL FACTORS

The performance of the test did not have a significant association with any of the following pre-analytical parameters in subjects that received a positive or negative result in the testing set: time from collection to processing (between 1-7 days), extent of hemolysis, and extent of lipemia. Samples were collected without any restrictions related to the time of day or the time of the dog's last feeding.

CONCLUSIONS

OncoK9 is a novel, blood-based multi-cancer early detection (MCED) test for the detection and characterization of cancer in dogs. Across all cancer-diagnosed subjects in the training and testing sets, OncoK9 detected cancer signal in at least one subject per cancer type across 30 distinct cancer types, demonstrating robust multi-cancer detection capabilities and suggesting utility as a non-invasive method for detecting a wide spectrum of canine cancers in screening and aid-in-diagnosis clinical use cases. Veterinarians now have access to a next-generation sequencing-based cancer detection test with performance comparable to state-of-the-art liquid biopsy tests in human medicine.

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FOOTNOTES

a. All subjects were enrolled under protocols that received institutional animal care and use committee (IACUC) or site-specific ethics approval, according to each site's requirements. All subjects were client-owned, and written informed consent was obtained from all owners.

b. In solid tumors, "localized/regional" refers to cancer that is limited to the organ of origin or to nearby lymph nodes, tissues, or organs; in lymphomas, this category refers to cancer that is limited to a single lymph node (Stage I) or multiple lymph nodes on one side of the diaphragm (Stage II).

c. In solid tumors, "disseminated/ metastatic" refers to cancer that has spread to areas of the body distant from the primary tumor; in lymphomas, this category refers to cancer that involves two or more lymph nodes on both sides of the diaphragm and/or one or more extra-nodal sites (Stages III, IV, and V). This category also included all non-lymphoma hematological cancer cases.

d. Undetermined refers to cases where there was insufficient evidence available to determine extent of disease, despite a complete cancer staging workup.

e. Includes B-cell and T-cell, as well as unclassified (non-phenotyped) lymphoma; excludes indolent lymphoma.

f. Includes cutaneous, intramuscular, abdominal visceral, and cardiac hemangiosarcoma.

g. Sensitivity: The percentage of cancer-diagnosed dogs who received a Cancer Signal Detected (positive) result; also known as the "detection rate".

h. Specificity: The percentage of cancer-free dogs who received a Cancer Signal Not Detected (negative) result. The false positive rate equals 1 minus specificity.