Clinical validation of a multi-cancer early detection (MCED) blood-based liquid biopsy test in dogs using next-generation sequencing (NGS)

Andi Flory, Daniel S. Grosu, Jason Chibuk, Lisa McLennan, Lauren E. Holtvoigt, Katherine M. Lytle, Jill M. Rafalko, Kristina M. Kruglyak, Ilya Chorny, Dana W. Y. Tsui

All authors are employed by PetDx, Inc., La Jolla, CA, USA

INTRODUCTION

Cancer is the leading cause of death in dogs, but 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, and prognosis is poor. Liquid biopsy methods that interrogate cancer-derived genomic alterations in cell-free DNA fragments in blood are being adopted for early cancer detection in human medicine and are now clinically available for use in veterinary medicine. **(Figure 1)** A noninvasive test for the early detection of cancer in dogs may lead to improved outcomes.

METHODS

As part of ongoing research and development programs at one commercial laboratory, blood samples were collected from over 1,700 canine subjects; and a subset was used for this analysis. A set of 200 subjects (54 cancer-diagnosed and 146 presumably cancer-free) was used to train the algorithms, and a set of 377 subjects (191 cancer-diagnosed and 186* presumably cancer-free) was used to validate the assay and establish test performance.

The cancer-diagnosed subjects in the training and validation sets were an all-comers cohort of cases with a definitive diagnosis of malignant tumor (cancer), representing over 40 cancer types and a full spectrum of cancer stages. The cancer-free subjects were presumed to be cancer-free due to no history of cancer and no suspicion of cancer on clinical evaluation at the time of study enrollment; other clinical conditions (trauma, sepsis, inflammation, etc.) were not considered exclusion criteria for the study, and fasting was not required prior to the blood collection.

Blood samples were subjected to DNA extraction, proprietary library preparation, and next-generation sequencing. Sequencing data were analyzed using an internally developed bioinformatics pipeline to detect genomic alterations associated with the presence of cancer. All data reviewers were blinded to the cancer status and type of cancer in these patients until after test results were issued.

Overall test performance was evaluated based on the 377 samples in the validation set, and secondary analyses were performed to determine the detection rate of the test in two subgroups of cancer-diagnosed subjects. The first subgroup analysis was performed for a cohort of 77 cancer-diagnosed dogs with any one of three of the most aggressive canine cancers (lymphoma, hemangiosarcoma, osteosarcoma). A second subgroup analysis was performed for a cohort of 114 cancer-diagnosed dogs with a cancer type from a predefined list of seven common cancers.[1,2] These seven cancers account for the majority of cancer mortality in dogs, specifically: lymphoma, hemangiosarcoma, osteosarcoma, soft-tissue sarcoma, mast cell tumor, mammary gland carcinoma, and malignant melanoma.

*[*The presumably-cancer free cohort initially included 188 dogs; however, two were excluded from analysis after receiving positive liquid biopsy results and, independently, definitive diagnoses of cancer.]*

RESULTS

The detection rate of the cell-free DNA-based liquid biopsy test for three of the most aggressive canine cancers (lymphoma, hemangiosarcoma, and osteosarcoma) was 81% (62/77). The detection rate for seven of the most common canine cancers (lymphoma, hemangiosarcoma, osteosarcoma, mast cell tumor, soft tissue sarcoma, mammary gland carcinoma, malignant melanoma) was 63% (72/114), and the overall 'multi-cancer' detection rate (across more than 40 types of cancer represented in the cancer-diagnosed all-comers cohort) was 48% (92/191). **(Figure 2)** A list of the cancer types detected by the assay (based on anatomical location) is shown in **Figure 3.** Of 188 samples from presumably cancer-free dogs, 180 tested negative ('putative true negatives') and 8 tested positive ('putative false positives', pFP). In 2 pFP cases, patients were diagnosed with cancer 6 and 7 months following blood collection, respectively, and were excluded from final performance analyses, resulting in a minimum test specificity of 97%, corresponding to a false positive rate of 3%. **(Figure 4)** The demographics of the subjects in the validation set are shown in **Table 1.**

Figure 1: Origins of cell-free DNA (cfDNA)[3]

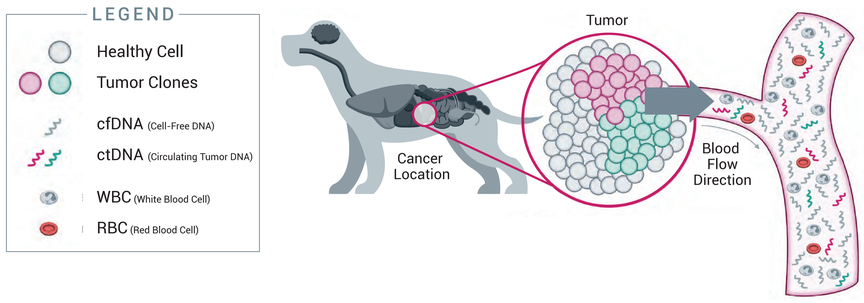


Figure 2: Detection rate (sensitivity) of the liquid biopsy assay in various subgroups of cancer-diagnosed subjects

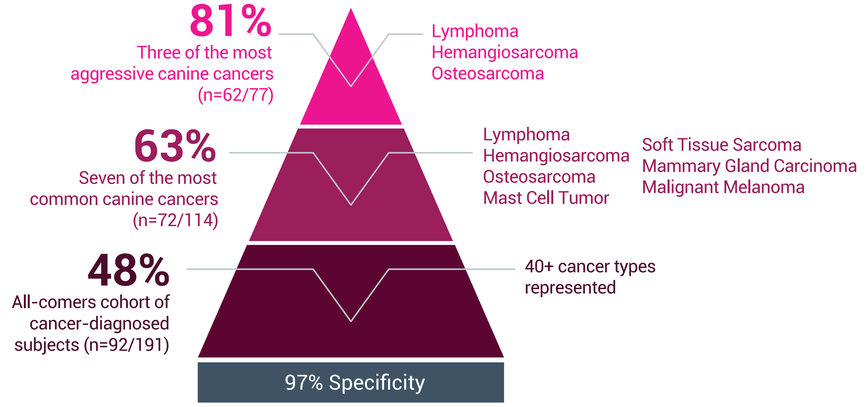
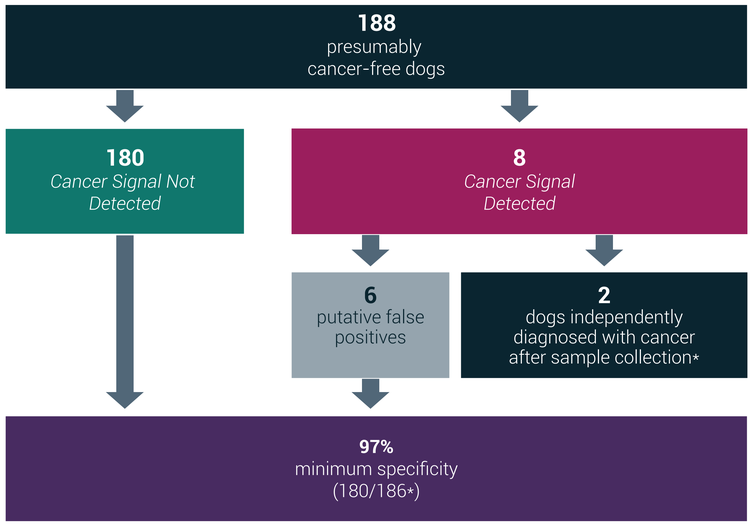


Figure 3: Cancer types detected by the cfDNA-based liquid biopsy assay, based on anatomical location

A	L	O
Abdominal Cavity	Lymphoma, Multicentric**	Oral Cavity
Anal Sac	Lymphoid Leukemia***	S
B	Liver	Salivary Gland
Bile Duct	Lung	Soft Tissue Sarcoma, Abdominal Visceral Organs
Bone	M	Soft Tissue Sarcoma, Head and Neck
H	Malignant Melanoma, Ungual	Soft Tissue Sarcoma, Trunk and Extremities
Heart Base	Mammary Gland	Spinal Cord
Hemangiosarcoma*	Mast Cell Tumor	T
K	N	Thyroid
Kidney	Nasal Cavity and Paranasal Sinuses	Transmissible Venereal Tumor (TVT)

Cancer cases were assigned a "cancer type" based on anatomical location; adapted from Withrow and MacEwen's Small Animal Clinical Oncology (Sixth Edition)[4] and from the American Joint Committee on Cancer (AJCC) Manual (Eighth Edition)[5]
* Includes subcutaneous, intramuscular, abdominal visceral, and cardiac
** Includes B-cell and T-cell as well as unclassified (non-phenotyped) lymphoma; excludes T-zone lymphoma
*** Includes acute and chronic lymphoid leukemia

Figure 4: Specificity calculations based on results of testing in presumably cancer-free dogs



*Two presumably cancer-free subjects were confirmed to have cancer after receiving positive liquid biopsy results and were excluded from analysis of test performance. The remaining 6 putative false positive cases are under surveillance.

Table 1: Demographics of presumably cancer-free and cancer-diagnosed subjects in the validation set (n=377)

		Presumably cancer-free subjects (n=186)	Cancer-diagnosed subjects (n=191)	p-value
Breed	Purebred	90	105	*p*=.201
	2 Breeds	15	13	*p*=.638
	3+ or unknown	81	73	*p*=.294
Sex	Male	90	103	*p*=.280
	Female	96	88	
Age	Range (years)	1 - 15*	2 – 15	
	Mean (years)	6.5	8.5	**p=<.0001****
	Median (years)	7	9	
Weight	Range (kg)	5.9 – 77.1	4.5 – 81.5	
	Mean (kg)	29.6	29.9	*p*=.393
	Median (kg)	29.5	29	

* n=184; 2 dogs excluded due to unknown age
** statistically significant

CONCLUSION

A novel, multi-cancer early detection (MCED) liquid biopsy test has demonstrated the ability to identify cancer-associated genomic alterations in canine patients, with sensitivity and specificity comparable to commercially available MCED testing options in humans,[6] and in some cases, many months prior to the onset of clinical signs. Liquid biopsy allows for detection of cancer with a simple blood draw, and can be employed in multiple clinical scenarios including as a cancer screening test and as an aid in diagnosis for cancer. Early detection and treatment of cancer are key determinants of optimal clinical outcomes.

KEY POINTS

1. Liquid biopsy using next-generation sequencing of cell-free DNA has the ability to detect cancer-associated genomic alterations in the blood of dogs with a variety of malignancies.
2. The liquid biopsy assay demonstrated an 81% detection rate for three of the most aggressive canine cancers, a 63% detection rate for seven of the most common canine cancers, and an overall 'multi-cancer' detection rate of 48% across over 40 cancer types included in the study.
3. False positive results are uncommon with liquid biopsy testing using cell-free DNA, and putative false positive results have been shown to be associated with early cancer detection in at least two subjects in the validation set.

References:
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