Orthologs of targetable human cancer somatic variants are detectable in canine cell-free DNA

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INTRODUCTION

Genotype-matched therapeutics are widely used in the treatment of human cancers. OncoKB (Oncology Knowledge Base) is the first FDA-recognized database that contains information about these targetable somatic variants and corresponding therapeutic agents, including levels of evidence.[1] **(Figure 1)** The clinical indications for therapeutic agents that target specific genomic alterations in people are typically further restricted to specific cancer types (referred to as "human cancer type indications"). In human cancer patients, variants targeted by these agents can be detected in tumor tissue, as well as in cell-free DNA through "liquid biopsy" testing. Given the high level of homology (>90%) across human and canine oncogenes and tumor suppressor genes **(Figure 2)**, it is not surprising that many of the human variants found in the OncoKB database have orthologs in the canine genome. This study was conducted to determine the feasibility of detecting such orthologs in the blood of cancer-diagnosed dogs.

METHODS

A representative cohort of 619 client-owned dogs with a variety of cancer diagnoses across all disease stages had pre-treatment plasma samples collected at the time of enrollment in a clinical validation study for a canine liquid biopsy test. A subset of these dogs also had matched tumor tissue available for analysis.

Blood samples (and tumor samples, when available) were subjected to DNA extraction and proprietary library preparation to enrich for genomic positions across more than 100 oncogenes and tumor suppressor genes, followed by next-generation sequencing. NGS data were analyzed using an internally developed bioinformatics pipeline to detect canine orthologs of human variants from the OncoKB database. Variants were called if they were observed in plasma cell-free DNA (cfDNA) at an allele frequency of at least 0.5%, with at least 6 unique supporting reads. Variants identified from plasma were subsequently genotyped in tissue without additional variant-level filtering.

Figure 1: OncoKB Human Therapeutic Levels of Evidence.[2]

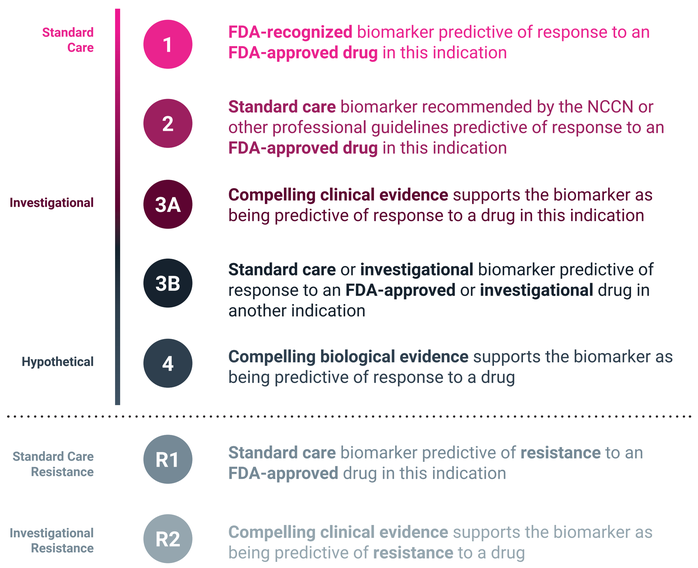


Figure 2: Percent similarity between human and canine orthologs at the protein level across the given classes where orthologs are mapped as 1:1. Cancer gene census (CGC) genes include oncogenes and tumor suppressor genes (TSG).[3]

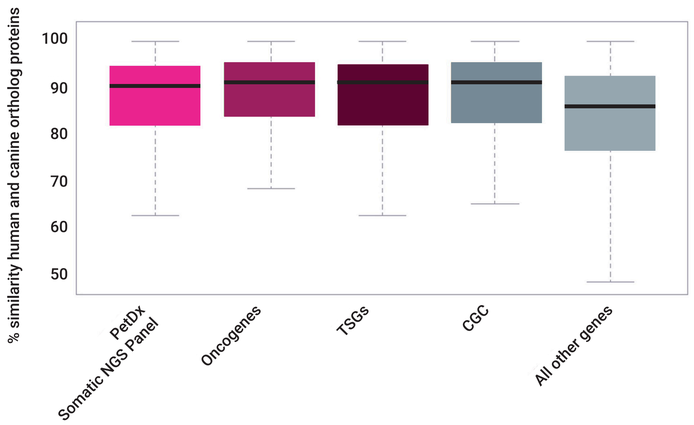
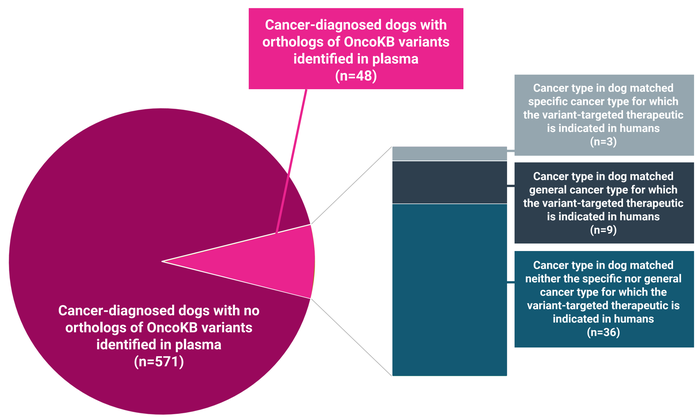


Figure 3: Results of testing for canine orthologs of OncoKB variants in pre-treatment plasma of 619 cancer-diagnosed dogs, and concordance of canine cancer type and human cancer type by genotype-matched indication across targeted therapeutic agents.



*Includes dogs with any solid tumor where the human indication for the drug was "All Solid Tumors".

Table 1: OncoKB orthologs identified in plasma cfDNA (+/- matched tissue) in cancer-diagnosed dogs. Table sorted alphabetically by gene and cancer type.

Canine Patient Number	Gene	Canine Variant (p.)	Human Variant (p.)	Variant Identified (cfDNA/Tissue)	Cancer Type(s) Identified in Canine Patient	Human Cancer Type Indication for Drug (OncoKB Level of Evidence)
R1	AKT1	E17K	E17K	cfDNA + tissue	Mammary Gland Carcinoma	• Breast Cancer (3) • Endometrial Cancer (3)
R2	BRAF	D582G	D594G	cfDNA	Osteosarcoma	• Langerhans Cell Histiocytosis (2) • Erdheim-Chester Disease (2) • Histiocytosis (3) • Rosai-Dorfman Disease (2)
	NRAS	Q61H	Q61H	cfDNA	Osteosarcoma	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3)
	PIK3CA	H1047R	H1047R	cfDNA	Osteosarcoma	• Breast Cancer (1)
	PTEN	R130Q	R130Q	cfDNA	Osteosarcoma	• All Solid Tumors (4)
R3	PTEN	D92G	D92G	cfDNA	Hemangiosarcoma	• All Solid Tumors (4)
R4	BRAF	G584R	G596R	cfDNA + tissue	Pulmonary Carcinoma	• Langerhans Cell Histiocytosis (2) • Erdheim-Chester Disease (2) • Histiocytosis (3) • Rosai-Dorfman Disease (2)
R5	BRAF	V588E	V600E	cfDNA	Urinary Bladder/ Urethra	• Melanoma (1) • Non-Small Cell Lung Cancer (1) • Glioma (2) • Astrocytoma (2) • Biliary Tract Cancer, NOS (3) • Colorectal Cancer (1, 2) • Ganglioma, Pleomorphic Xanthoastrocytoma, Pilocytic Astrocytoma (2) • Encapsulated Glioma (2) • Hairy Cell Leukemia (2) • Anaplastic Thyroid Cancer (1)
R6	EGFR	L792R	L858R	cfDNA	Lymphoma, Intermediate to Large Cell	• Non-Small Cell Lung Cancer (1, 3)
R7	HRAS	Q61R	Q61R	cfDNA + tissue	Pulmonary Carcinoma	• Head and Neck Squamous Cell Carcinoma (3) • Urothelial Carcinoma (3)
R8	HRAS	Q61L	Q61L	cfDNA	Sebaceous Adenocarcinoma	• Squamous Cell Carcinoma (3) • Urothelial Carcinoma (3)
R9	KIT	N508I	N505I	cfDNA	Mast Cell Tumor	• Gastrointestinal Stromal Tumor (1)
R10	KIT	Y822D	Y823D	cfDNA	Mast Cell Tumor	• Gastrointestinal Stromal Tumor (1, 2, 4, R2)
R11	KIT	V558A	V559A	cfDNA	Mast Cell Tumor	• Gastrointestinal Stromal Tumor (1) • Melanoma (2)
R12	KRAS	A153T	A59T	cfDNA	Anal Sac	• Colorectal Cancer (R1) • All Solid Tumors (4) • Histiocytosis (3)
	KRAS	S159N	S65N	cfDNA	Anal Sac	• Colorectal Cancer (R1) • All Solid Tumors (4) • Histiocytosis (3)
	PTEN	C136Y	C136Y	cfDNA	Anal Sac	• All Solid Tumors (4)
	PTEN	V133I	V133I	cfDNA	Anal Sac	• All Solid Tumors (4)
R13	KRAS	G106D	G12D	cfDNA	Histiocytic Sarcoma	• Colorectal Cancer (R1) • Histiocytosis (3) • All Solid Tumors (4)
R14	KRAS	A240T	A146T	cfDNA	Lymphoma, Intermediate to Large Cell	• Colorectal Cancer (R1) • Histiocytosis (3) • All Solid Tumors (4)
R15	KRAS	G107D	G13D	cfDNA	Lymphoma, Intermediate to Large Cell	• Colorectal Cancer (R1) • All Solid Tumors (4) • Histiocytosis (3)
R16	KRAS	G107D	G13D	cfDNA	Lymphoma, Intermediate to Large Cell	• Colorectal Cancer (R1) • Histiocytosis (3) • All Solid Tumors (4)
R17	KRAS	G107D	G13D	cfDNA + tissue	Lymphoma, Intermediate to Large Cell	• Colorectal Cancer (R1) • All Solid Tumors (4) • Histiocytosis (3)
R18	KRAS	K211N	K117N	cfDNA	Lymphoma, Intermediate to Large Cell	• Colorectal Cancer (R1) • Histiocytosis (3) • All Solid Tumors (4)
R19	KRAS	G107D	G13D	cfDNA	Malignant Melanoma (Ungual)	• Colorectal Cancer (R1) • All Solid Tumors (4) • Histiocytosis (3)
R20	KRAS	G106V	G12V	cfDNA	Pulmonary Carcinoma	• Colorectal Cancer (R1) • Histiocytosis (3) • All Solid Tumors (4)
R21	KRAS	G106A	G12A	cfDNA	Squamous Cell Carcinoma (Nasal Cavity)	• Colorectal Cancer (R1) • All Solid Tumors (4) • Histiocytosis (3)
R22	NRAS	Q61H	Q61H	cfDNA + tissue	Bone, Osteosarcoma	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3)
R23	NRAS	Q61L	Q61L	cfDNA	Hemangiosarcoma	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3)
R24	NRAS	Q61R	Q61R	cfDNA	Hemangiosarcoma	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3) • Colorectal Cancer (R1)
R25	NRAS	G13R	G13R	cfDNA	Hemangiosarcoma; Pancreatic Carcinoma; Hepatocellular Carcinoma	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3)
R26	NRAS	G13D	G13D	cfDNA	Lymphoma, Intermediate to Large Cell	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3)
R27	NRAS	G12S	G12S	cfDNA + tissue	Malignant Melanoma	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3)
R28	NRAS	G12D	G12D	cfDNA + tissue	Soft Tissue Sarcoma	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3)
R29	NRAS	Q61L	Q61L	cfDNA + tissue	Soft Tissue Sarcoma	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3)
R30	NRAS	G13D	G13D	cfDNA	Transmissible Venereal Tumor	• Melanoma (3, 4) • Colorectal Cancer (R1) • Histiocytosis (3) • Thyroid Cancer (3)
R31	PIK3CA	H1047R	H1047R	cfDNA + tissue	Anal Sac	• Breast Cancer (1)
R32	PIK3CA	C420R	C420R	cfDNA + tissue	Hemangiosarcoma	• Breast Cancer (2)
R33	PIK3CA	C420R	C420R	cfDNA	Hemangiosarcoma	• Breast Cancer (2)
R34	PIK3CA	H1047R	H1047R	cfDNA	Hemangiosarcoma	• Breast Cancer (1)
R35	PIK3CA	E542K	E542K	cfDNA	Liver	• Breast Cancer (1)
R36	PIK3CA	E542K	E542K	cfDNA	Lymphoma, Intermediate to Large Cell	• Breast Cancer (1)
R37	PIK3CA	H1047Y	H1047Y	cfDNA	Lymphoma, Intermediate to Large Cell	• Breast Cancer (1)
R38	PIK3CA	G118D	G118D	cfDNA + tissue	Oral Cavity	• Breast Cancer (2)
R39	PIK3CA	E542K	E542K	cfDNA	Osteosarcoma	• Breast Cancer (1)
	PIK3CA	G118D	G118D	cfDNA	Osteosarcoma	• Breast Cancer (2)
	PIK3CA	H1047L	H1047L	cfDNA + tissue	Osteosarcoma	• Breast Cancer (1)
	PIK3CA	R38C	R38C	cfDNA + tissue	Osteosarcoma	• Breast Cancer (2)
R40	PIK3CA	E453K	E453K	cfDNA	Soft Tissue Sarcoma	• Breast Cancer (2)
R41	PIK3CA	E600K	E600K	cfDNA	Transmissible Venereal Tumor	• Breast Cancer (2)
R42	PTEN	C136Y	C136Y	cfDNA	Lymphoma, Intermediate to Large Cell	• All Solid Tumors (4)
	PTEN	V133I	V133I	cfDNA	Lymphoma, Intermediate to Large Cell	• All Solid Tumors (4)
R43	PTEN	K125E	K125E	cfDNA	Lymphoma, Intermediate to Large Cell	• All Solid Tumors (4)
R44	PTEN	R173H	R173H	cfDNA	Lymphoma, Intermediate to Large Cell	• All Solid Tumors (4)
R45	PTEN	C136Y	C136Y	cfDNA	Mast Cell Tumor	• All Solid Tumors (4)
	PTEN	V133I	V133I	cfDNA	Mast Cell Tumor	• All Solid Tumors (4)
R46	PTEN	C136Y	C136Y	cfDNA	Mast Cell Tumor	• All Solid Tumors (4)
	PTEN	V133I	V133I	cfDNA	Mast Cell Tumor	• All Solid Tumors (4)
R47	PTEN	C136Y	C136Y	cfDNA	Nasal Cavity and Paranasal Sinuses (Chondrosarcoma)	• All Solid Tumors (4)
	PTEN	V133I	V133I	cfDNA	Nasal Cavity and Paranasal Sinuses (Chondrosarcoma)	• All Solid Tumors (4)
R48	PTEN	C136Y	C136Y	cfDNA	Rhabdomyosarcoma	• All Solid Tumors (4)
	PTEN	V133I	V133I	cfDNA	Rhabdomyosarcoma	• All Solid Tumors (4)

RESULTS

Analysis of pre-treatment plasma identified canine orthologs of OncoKB variants in 7.8% (48/619) of subjects, and in 25% (12/48) of these patients, the dog had a cancer type that matched the human indication for a therapeutic agent targeting the detected variant. This analysis included cases where the OncoKB cancer type was listed as "All Solid Tumors" and the canine diagnosis was a solid tumor (e.g., hemangiosarcoma, nasal cavity tumor, anal sac adenocarcinoma, rhabdomyosarcoma, etc.). It should be noted that certain cancer types, such as mast cell tumors, anal sac adenocarcinomas, and transmissible venereal tumors do not have direct biological counterparts in humans, so it is uncertain whether a drug targeting "All Solid Tumors" would be appropriate to use in a canine patient with these cancer types, despite their classification as solid tumors.

When the OncoKB cancer type "All Solid Tumors" was excluded, and analysis focused on specifically matched cancer types, 3 of the 48 dogs (6.3%) had a specific cancer type that matched the human indication for a therapeutic agent targeting the detected variant, including one case of histiocytic sarcoma, one case of malignant melanoma, and one case of mammary gland carcinoma. **(Figure 3)** A full list of the OncoKB orthologs identified in canine patients can be found in **Table 1**.

Of the 48 dogs in which an OncoKB ortholog was identified in plasma, 44% (21/48) had matched tumor tissue available for analysis. In 12 of the 21 subjects (54%), the variant(s) detected in plasma were also present in the matched tissue. The cases in which matched tumor tissue was positive for the variant identified in cfDNA are listed in the column titled "Variant Identified (cfDNA/ Tissue)" of **Table 1**.

CONCLUSION

This study demonstrates that non-invasive detection of canine orthologs of targetable human cancer somatic variants is technically feasible in dogs through next-generation sequencing-based "liquid biopsy" using a simple blood draw; however, it is currently unclear how human databases can be used to inform targeted treatment decisions in dogs. When orthologs of targetable variants were identified in the plasma of dogs, the cancer type in the dog generally matched the human indication for a therapeutic agent targeting the detected variant in about 25% of cases; and only in 6% of cases there was a match at the level of a specific cancer type across the two species. Even in humans, the response to targeted treatments can be highly variable depending on the cancer type in the patient[4] ; and less than 20% of human cancer patients are estimated to harbor somatic variants that are currently targetable by FDA-approved therapeutic agents[5]. Furthermore, in dogs, there is a scarcity of large-scale safety and efficacy data to support the use of targeted therapies at this time. As the evidence base for the utility of such therapies in dogs becomes available, it is expected that next-generation sequencing-based liquid biopsy will play an important role in targeted therapy selection for canine cancer patients, mirroring recent trends in human oncology.[6,7]

Cancer type in dog matched specific cancer type for which the variant-targeted therapeutic is indicated in humans

Cancer type in dog matched general cancer type for which the variant-targeted therapeutic is indicated in humans; includes dogs with any solid tumor where the human indication for the drug was "All Solid Tumors"

REFERENCES

[1] Chakravarty D, Gao J, Phillips S, et al. OncoKB: A Precision Oncology Knowledge Base. JCO Precis Oncol 2017;2017:1–16.

[2] Memorial Sloan Kettering Cancer Center. OncoKB Therapeutic Level of Evidence v2. Available at: https://www.oncokb.org/levels. Accessed January 4, 2023.

[3] Chorny I, Kruglyak KM, Tynan JA, et al. Comparative oncology analysis of canine cancer by tumor and liquid biopsy testing for biomarker and therapeutic discovery in humans and dogs. Poster presented at the American Association for Cancer Research (AACR) Annual Meeting 2022; Apr 8-13, 2022; New Orleans, LA.

[4] Kopetz S, Desai J, Chan E, et al. Phase II Pilot Study of Vemurafenib in Patients With Metastatic BRAF-Mutated Colorectal Cancer. J Clin Oncol 2015;33:4032–4038.

[5] Haslam A, Kim MS, Prasad V. Updated estimates of eligibility for and response to genome-targeted oncology drugs among US cancer patients, 2006-2020. Ann Oncol. 2021;32(7):926–32.

[6] Husain H, Velculescu VE. Cancer DNA in the Circulation: The Liquid Biopsy. JAMA. 2017;318(13):1272–4.

[7] Ignatiadis M, Sledge GW, Jeffrey SS. Liquid biopsy enters the clinic — implementation issues and future challenges. Nat Rev Clin Oncol. 2021;1–16.

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