SearchLight DNA™ Clinician Report

Received:09/28/21 Reported:10/08/21

Pet:	Owner:	Species:	Breed:	Sex:	Age:	Site:
		Canine	Labradoodle	Female	9y	Right side of face, left flank

Diagnosis:

Poorly Differentiated Malignant Tumor

SearchLight DNA Overview

Biomarkers Identified: 7

ATM	SMARCB1
CDKN2B	TP53
CHEK2	
NF2	
PTEN	

Number of Clinical Trials:

- **This Cancer Type:** 0
- **General Cancer:** 15

7 Diagnostic Biomarkers

3 Prognostic Biomarkers

2 Matching Drugs: Olaparib, Everolimus

Sample QC Metrics
Specimen Type: FFPE Slides
Tumor Content (>20%): 70%
Mean Target Coverage (>200x): 227x

SearchLight DNA Summary

This test evaluated 120 cancer genes in the patients tumor sample. The ABCB1-1Δ (MDR1-1Δ) mutation was not detected, supporting that patient is unlikely to experience ABCB1-1Δ-related adverse effects of chemotherapy. 7 alterations were identified of potential clinical significance for cancer diagnosis, prognosis or treatment.

Limited genomic information is available for most canine sarcoma subtypes. However, integrated review of the genomic data for patient's sample, as well as clinical history and pathology reports is consistent with the general diagnosis of sarcoma. Specifically, deep deletion of CDKN2B as well as copy number losses of TP53 and PTEN are common events in both canine and human sarcomas. In addition, copy number losses in ATM, CHEK2, and SMARCB1 have also been found in human sarcomas. Notably, SMARCB1 deletions are enriched in human epithelioid sarcomas, but no data is yet available in dogs.

Therapeutic Biomarkers

Treatment Options Based on Mutations

Drug	Mutation	Available for dogs	Used in humans
Olaparib	ATM Copy Number Loss	Yes[C]	Yes[C]
Everolimus	PTEN Copy Number Loss	-----	Yes[A]

Drug Resistance-Associated Biomarkers

Drug	Mutation
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Pharmacogenomic Biomarkers

Gene	Mutation
ABCB1	No mutation

Diagnostic Biomarkers

Gene	Mutation	Described in: Canine cancer	Human cancer
ATM	Copy Number Loss	-----	Yes[B]
CDKN2B	Copy Number Loss	Sarcoma[A], Pulmonary Adenocarcinoma[B]	Yes[A]
CHEK2	Copy Number Loss	-----	Yes[B]
NF2	Copy Number Loss	-----	Yes[C]
PTEN	Copy Number Loss	Mast Cell Tumor[C]	Yes[D]
SMARCB1	Copy Number Loss	-----	Yes[A]
TP53	Copy Number Loss	Osteosarcoma[D], Hemangiosarcoma[B], Histiocytic Sarcoma[B]	Yes[D]

Prognostic Biomarkers

Gene	Mutation	Negative Prognostic Factor in: Canine cancer	Human cancer
CDKN2B	Copy Number Loss	Pulmonary Adenocarcinoma[B]	Yes[B]
PTEN	Copy Number Loss	-----	Yes[A]
TP53	Copy Number Loss	Mast Cell Tumor[D]	Yes[B]

Evidence Level Key

A Validated biomarker - Proven biomarker with wide consensus and whose use is included in professional guidelines

B Clinical evidence - Biomarker with consensus from experts in the field with data obtained from large, well powered studies

C Case studies - Biomarker suggested by data from one or more individual case reports from clinical journals

D Preclinical evidence - Biomarker suggested by data from in vivo or in vitro models

Mutation Summaries

Gene:	ATM	Mutation:	Copy Number Loss

Roles in this case: ⊕ Diagnostic 💊 Therapeutic

Variant Summary:
The ATM tumor suppressor is frequently inactivated via deletion or truncating mutation in human T-cell prolymphocytic leukemia and, via its role in familial ataxia-telangiectasia, in hereditary leukemia, lymphoma, medulloblastoma, and glioma. It has also been found to be deleted in canine prostate carcinoma. Disruption of ATM is predicted to impair its tumor suppressive function. Loss of ATM is associated with defects in DNA repair in cancer.

Detailed Summary:
Please see **Link** for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene:	CDKN2B	Mutation:	Copy Number Loss

Roles in this case: ⊕ Diagnostic ⋀ Prognostic

Variant Summary:
CDKN2A and the adjacent CDKN2B genes (CDKN2A/B) are tumor suppressors commonly deleted in human glioblastoma (56%), mesothelioma (45%), esophageal cancer (39%), bladder cancer (32%), melanoma (31%), head and neck carcinoma (31%), pancreatic cancer (28%), diffuse large B-cell lymphoma (27%), lung squamous cell carcinoma (26%), lung adenocarcinoma (17%), cholangiocarcinoma (17%), sarcoma (15%), stomach cancer (11%), low grade glioma (11%), adrenocortical carcinoma (7%), liver cancer (6%), and other cancers. CDKN2A/B is also frequently mutated or deleted in canine cancers including malignant melanoma (~68%), histiocytic sarcoma (~63%), osteosarcoma (~42-70%), T-cell lymphoma (~40%), pulmonary adenocarcinoma (~40%), urothelial carcinoma (~26%), head and neck squamous cell carcinoma (~25%), hemangiosarcoma (~22-28%), KIT-mutant mast cell tumors (~21%), and glioma (~10%). CDKN2A/B deletion leads to loss of functional protein (the p16 and p14 tumor suppressors) and disruption of the tumor suppressive effects of these proteins. In human cancers, CDKN2A/B deletion has been associated with poor prognosis in sarcomas. In dogs, CDKN2A/B deletion and/or promoter methylation has also been associated with high grade in lymphoma.

Detailed Summary:
Please see **Link** for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene:	NF2	Mutation:	Copy Number Loss

Roles in this case: ⊕ Diagnostic

Variant Summary:
NF2 is mutated in human meningioma, acoustic neuroma, and renal cancer, typically through deletions and inactivating point mutations. It is also involved in neurofibromatosis type 2 and germline variation is associated with meningioma and acoustic neuroma predisposition. NF2 has been shown to be deleted in canine mammary cancer.

Detailed Summary:
Please see **Link** for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Mutation Summaries

Gene:	CHEK2	Mutation:	Copy Number Loss

Roles in this case: ⊕ Diagnostic   🧪 Therapeutic

Variant Summary:
CHEK2 inactivating mutations (typically truncating point mutations) are associated with human sporadic and familial breast cancer. CHEK2 deletions are seen at low frequency in human adrenocortical carcinoma (3%), thymoma (3%), and other cancers. CHEK2 deletions have also been identified in ~9% of canine mammary tumors. These deletions lead to loss of functional CHEK2 protein and disruption of its tumor suppressive effects and has been associated with DNA repair defects in cancer. CHEK2 deletion has also been associated with response to the PARP inhibitor, olaparib, in human prostate cancer.

Detailed Summary:
Please see **Link** for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene:	PTEN	Mutation:	Copy Number Loss

Roles in this case: ⊕ Diagnostic   ⤳ Prognostic   🧪 Therapeutic

Variant Summary:
The distal end of chromosome 26 including the PTEN gene is frequently deleted in canine cancers. PTEN is commonly mutated through deletion or inactivating mutation in osteosarcoma (~46-63%), histiocytic sarcoma (~41-56%), T cell lymphoma (~10%,enriched at 25% in Boxers), hemangiosarcoma (~4-10%), pulmonary adenocarcinoma (~6%), mammary gland tumors (~5%), oral malignant melanoma (~5%), glioma (~2%), and chronic monocytic leukemia (one case report). PTEN copy number loss is associated with worse prognosis in canine mast cell tumors, and in human breast cancer, prostate cancer, and lung cancer. Loss of PTEN has been associated with sensitivity to mTOR inhibition in human prostate cancer.

Detailed Summary:
Please see **Link** for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene:	SMARCB1	Mutation:	Copy Number Loss

Roles in this case: ⊕ Diagnostic

Variant Summary:
SMARCB1 is located on canine chromosome 26. SMARCB1 is disrupted in a subset of human cancers, typically by mutation or copy number loss. The spectrum of SMARCB1-deficient human tumors includes epithelioid sarcoma, small cell carcinoma of the ovary, sinonasal carcinoma, papillary renal cell carcinoma, and others. SMARCB1 deletion is predicted to result in impairment or loss of SMARCB1 tumor suppressive function.

Detailed Summary:
Please see **Link** for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Mutation Summaries

Gene:	TP53	Mutation:	Copy Number Loss

Roles in this case: ✚ Diagnostic ⚲ Prognostic

Variant Summary:
TP53 is the most commonly mutated tumor suppressor gene in canine and human cancers. TP53 is commonly mutated through deletion or inactivating mutation in canine osteosarcoma (~53%-71%), hemangiosarcoma (~60%), malignant melanoma (~19%), B-cell and T-cell lymphoma (~15.6% and 4.9-6%), mast cell tumors (10-15%), pulmonary adenocarcinoma (~12.5%), glioma (~12%), histiocytic sarcoma (~9%), mammary gland tumors (~4-8%), squamous cell carcinoma of the skin (~4%), and cancer cell lines (33%). TP53 loss is also associated with poor prognosis in many human cancers as well as with higher-risk disease in canine mast cell tumors.

Detailed Summary:
Please see **Link** for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Clinical Trials Summary

Clinical Trial for this tumor type	Location	Website
UW-SVM Solid Tumor - Dogs with any cancer (except lymphoma or leukemia): Evaluation of the antitumor effect of administration of the dog's own activated immune (t) cells	University of Wisconsin Madison, WI	Link
MU-CVM Solid Tumor - Hiltonol(R)-poly-ICLC	University of Missouri Columbia, MO	Link
UCD-SVM Solid Tumor - Evaluating long-acting local anesthetic on pain control in dogs following front leg amputation	University of California-Davis Davis, CA	Link
UCD-SVM Solid Tumor - Assessing a new therapy for dogs with cancer	University of California-Davis Davis, CA	Link
UIUC-CVM Solid Tumor - Preclinical assessment of an oral p97 inhibitor, CB-5339, in tumor-bearing dogs	University of Illinois Champaign-Urbana, IL	Link

Other Clinical Trials that may be applicable

10 identified   See link for details

Variants of Unknown Significance

The following variants were detected in _____ tumor sample. These variants are considered variants of uncertain significance, meaning the functional impact of the alteration on gene function is unknown or the role of the mutation in tumor diagnosis, prognosis, or treatment is unknown. Future research may reveal a role for the mutations in cancer.

• ASXL1(Copy Number Loss)
• FANCG(Copy Number Loss)
• FLCN(Copy Number Loss)

References

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Additional Supporting Information

1. Alteration frequencies in human cancers are derived from COSMIC https://cancer.sanger.ac.uk/ and the TCGA pan-cancer cohort, as accessed through cBioPortal https://www.cbioportal.org/

2. Gene summaries are based on gene descriptions provided by the National Library of Medicine and National Center for Biotechnology Information https://www.ncbi.nlm.nih.gov/gene

3. Mealey et al. ABCB1-1Delta polymorphism can predict hematologic toxicity in dogs treated with vincristine. J Vet Intern Med (2008). https://pubmed.ncbi.nlm.nih.gov

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Genes Evaluated by SearchLight™ DNA

SearchLight DNA™ detects multiple types of gene mutations:

- Single nucleotide variants, small nucleotide insertions and deletions (SNVs) occurring in selected commonly mutated regions in oncogenes ("Selected Exons") or in any coding region of a tumor suppressor gene ("All Coding Exons").
- Copy number variants (CNVs) including copy number gains encompassing oncogenes and copy number losses encompassing tumor suppressor genes.
- Internal tandem duplications (ITDs) occurring in oncogenes.
- Pharmacogenomic variants in genes that regulate drug processing.

Panel 1

	ABCB1	AKT1	AKT3	ALK	APC	ARAF	ARID1A	ASXL1	ATM	ATR	ATRX	BAP1	BRAF	BRCA1	BRCA2	BTK	CALR	CBL	CCND1	CCND2	CCND3	CCNE1	CDK12	CDK4
SNV Selected Exons		●	●	●		●							●			●	●	●	●					
SNV All Coding Exons					●		●	●	●		●			●	●							●		
CNV		●		●	●		●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●
ITD																								
Pharmacogenomic	●																							

Panel 2

	CDK6	CDKN2A	CDKN2B	CHEK2	CRKL	CSF3R	CTNNB1	DDR2	DNMT3A	EGFR	ERBB2	ERRFI1	ESR1	EZH2	FANCA	FANCC	FANCG	FANCL	FBXW7	FGF3	FGFR1	FGFR2	FGFR3	FLCN
SNV Selected Exons					●	●	●	●		●	●		●	●							●	●	●	
SNV All Coding Exons		●	●	●					●			●						●						●
CNV	●	●	●	●	●				●	●	●				●	●	●	●	●	●	●	●	●	●
ITD																								
Pharmacogenomic																								

Panel 3

	FLT3	FOXL2	GNAQ	GNAS	GNB1	H3F3A	HRAS	IDH1	IDH2	IKZF1	JAK1	JAK2	KDR	KIT	KMT2D	KRAS	MAP2K1	MAP2K2	MAPK1	MDM2	MDM4	MEN1	MET	MLH1
SNV Selected Exons		●	●	●	●	●	●	●	●		●	●	●	●		●	●	●	●				●	
SNV All Coding Exons																						●		●
CNV									●			●	●	●		●	●	●	●	●			●	
ITD	●											●												
Pharmacogenomic																								

Panel 4

	MSH2	MSH3	MSH6	MTOR	MYC	MYCN	MYD88	NF1	NF2	NFE2L2	NOTCH1	NPM1	NRAS	NT5C2	PALB2	PDGFRA	PIK3CA	PIK3R1	PMS2	POLD1	POLE	PTCH1	PTEN	PTPN11
SNV Selected Exons				●	●		●			●		●	●			●	●						●	
SNV All Coding Exons	●	●	●					●	●					●	●			●	●	●	●	●		
CNV	●	●	●	●	●	●		●	●		●		●	●		●	●		●	●	●		●	
ITD																								
Pharmacogenomic																								

Panel 5

	RAC1	RAF1	RB1	REL	RET	RICTOR	RUNX1	SDHB	SDHD	SETD2	SF3B1	SMAD4	SMARCA4	SMARCB1	SMO	STAT3	STK11	TP53	TRAF3	TSC1	TSC2	U2AF1	VEGFA	VHL
SNV Selected Exons	●	●		●	●			●								●	●							
SNV All Coding Exons			●				●	●	●		●	●	●	●			●	●	●	●	●			●
CNV		●	●	●	●	●	●		●			●	●		●	●	●	●		●	●		●	
ITD																								
Pharmacogenomic																								

Assay Description

SearchLight DNA™ detects multiple types of gene mutations:

SearchLight DNA™ is a Next Generation Sequencing targeted tumor-only assay that provides for the detection of single nucleotide variants (SNVs), small nucleotide insertions and deletions (indels), copy number variants (CNVs), internal tandem duplications (ITDs), and polymorphisms in tumor tissue. Genomic DNA is extracted from the patient's tumor samples and the isolated DNA is then prepared using a custom hybrid capture panel (Agilent). Library preparation includes shearing, purification, adaptor ligation and PCR amplification. Libraries are then clustered on a flow cell and sequenced using the Illumina MiSeq or NextSeq. Sequence data are analyzed using validated bioinformatics tools (SearchLight DNA™ Pipeline 1.2) and canine polymorphism databases. The reference genome assembly used for alignment is CanFam 3.1. Each tumor's candidate cancer-specific mutations are queried against Vidium's proprietary knowledgebase which contains thousands of canine cancer biomarker associations derived from primary peer-reviewed literature to identify potential pharmacogenomic, diagnostic, prognostic, and therapeutic associations. Additionally, this knowledgebase contains human cancer biomarker associations inferred via genomic and proteomic alignments and conservation scores from the Clinical Interpretation of Variants in Cancer (CIViC version 05/01/20) and Catalogue of Somatic Mutations in Cancer (COSMIC version 91) databases. ABCB1 germline genotype is determined based on tumor-only sequencing. SNVs are reported when present at ≥ 3% allele fraction. Allele fractions are dependent on tumor purity. Tumor purity is not taken into account when calculating allele fractions. Reported CNVs (gains/losses) are identified based on comparison to a copy number baseline generated from normal tissues across major breed clades and tissue types. Reported CNVs may be focal, arm-level, or chromosome-level. ITDs are reported only for KIT and FLT3 in selected exons. Pharmacogenomic polymorphisms are reported only for ABCB1 (also known as MDR1). Indeterminate results may occur due to poor sample quality or sequencing coverage. Mean target coverage for tumor sample DNA is ≥ 200x (unique reads) and ≥ 89% of target bases bear ≥ 100x coverage.

Limitations

Samples with a tumor content less than 30% may have reduced sensitivity and lead to false negative results. It is also possible that the sample contains a mutation below our established limit of detection or in a genetic region not included in our assay. Alterations present in repetitive or high GC content region or non-coding areas may not be detected. Indels larger than 40bp may not be detected. Copy number signal relative to background noise inherent in DNA from FFPE samples may affect sensitivity of reporting CNV gains/losses. The lack of a variant call does not necessarily indicate the absence of a variant since technical limitations to acquire data in some genetic regions may limit assay detection. ABCB1 germline genotype is inferred from tumor-only sequencing and it remains possible, though unlikely, that either ABCB1 loss of heterozygosity in the tumor or somatic acquisition of an ABCB1 mutation could interfere with accurate genotyping.

Disclaimers

This test was developed, and performance characteristics determined, by Vidium Animal Health. This test has not been approved by the U.S. FDA. The FDA has determined that such clearance or approval for veterinary diagnostics is not necessary. This test is used for clinical purposes for veterinary patients. It should also be noted that the data interpretations are based on our current understanding of genes and variants and are current as of the report date. Alterations are listed alphabetically, and not in order of strength of evidence or appropriateness for the patient's disease. When the report does identify variants with therapeutic implications, this does not promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient, and the selection of any drug for patient treatment is done at the discretion of the treating veterinarian. These treatment options are based solely on published biomarker associations and do not include dosing, safety, or combinatorial guidelines. Please refer to drug labeling, published literature, and safety data for warnings, precautions, and dosing guidelines. Use caution when combining multiple drugs and be aware of potential drug interactions. Genomic alterations should be considered in the context of the patient's history, risk factors and any previous genomic testing. Variants of Unknown Significance (VUS) may be associated with potential therapies in the future. Vidium does not update reports or send notification regarding reclassification of these alterations. Vidium Animal Health's services, including but not limited to the results contained in this report, are governed by Vidium's Terms & Conditions, which are available by email by requesting them at **vidiuminfo@tgen.org**.

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Electronically Signed by:

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