SearchLight DNA® Clinician Report

[redacted]

Phone Number: [redacted]
External Lab Reference No: [redacted]

Accession: SL23-000377
Received: 05/12/23
Reported: 05/19/23

Pet:	Owner:	Species:	Breed:	Sex:	Age:
[redacted]	[redacted]	Canine	Papillon	F/S	13 y

Site: tongue incisional Bx

Diagnosis: Malignant Discrete Cell Tumor

SearchLight DNA Overview

2 Biomarker(s) identified in the following genes:

NF1

Sample QC Metrics

Specimen Type: FFPE Slides

Tumor Content (>20%): 80%

Mean Target Coverage (>200x): 203x

Number of Clinical Trials:

- **This Cancer Type:** 18
- **General Cancer:** 18

2 Diagnostic Biomarkers

0 Prognostic Biomarkers

2 Matching Drugs: selumetinib, trametinib

SearchLight DNA Summary

An integrated review of the genomic data, as well as clinical history and pathology reports, is consistent with a diagnosis of melanoma. Specifically, inactivating mutations in NF1 (e.g. Arg383* and Arg1186* as found in this case), have been commonly found in canine melanoma. However, they have also been sporadically detected in histiocytic sarcoma and osteosarcoma, based on internal data.

Notably, we identified mutations with therapeutic and/or prognostic associations, as described on page 2. Trametinib is available through veterinary compounding pharmacies. A monograph describing published data on the use of this agent in dogs is available upon request, or you can find it on our website (https://vidiumah.com/monographs/).

This test evaluated 120 cancer genes in the submitted sample. The ABCB1-1delta (MDR1-1delta) mutation was not detected, indicating that the patient is unlikely to experience the ABCB1-1delta-related adverse effects of chemotherapy.

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Pet Name: [redacted]

Therapeutic Biomarkers

Treatment Options Based on Mutations

Drug	Mutation	Available for dogs	Used in humans
selumetinib	NF1 p.Arg1186*	No	Yes
selumetinib	NF1 p.Arg383*	No	Yes
trametinib	NF1 p.Arg1186*	Yes	Yes
trametinib	NF1 p.Arg383*	Yes	Yes

Drug Resistance-Associated Biomarkers

Drug	Mutation
-	-

Pharmacogenomic Biomarkers

Gene	Mutation
ABCB1	No ABCB1-1Δ Mutation

Diagnostic Biomarkers

		Described in:	
Gene	Mutation	Canine cancer	Human cancer
NF1	p.Arg1186*	Mammary Cancer , Melanoma	Yes
NF1	p.Arg383*	Mammary Cancer , Melanoma	Yes

Prognostic Biomarkers

		Negative Prognostic Factor in:	
Gene	Mutation	Canine cancer	Human cancer
-	-	-	-

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

Pet Name: [redacted]

Gene	ABCB1	Mutation	No ABCB1-1Δ Mutation

Variant Summary:

A polymorphism (referred to as ABCB1-1delta) occurs in a subset of dog breeds, including many herding breeds. The ABCB1-1delta polymorphism is a 4-base pair deletion that causes a shift in the reading frame that results in premature truncation of P-glycoprotein and loss of P-glycoprotein function. Dogs that are homozygous or heterozygous for this polymorphism can experience increased toxicity for chemotherapeutic agents that are substrates for ABCB1, such as doxorubicin, vincristine, and vinblastine. Dogs without this polymorphism (non-mutant) show standard susceptibility to chemotherapy-associated adverse effects, and a dosing adjustment based on ABCB1 status is not needed. (Mealey et al. J Vet Intern Med 2008; Mealey et al. Vet Clin North Am Small Anim Pract 2013)

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	NF1	Mutation	p.Arg1186*

Roles in this case: Diagnostic Therapeutic

Variant Summary:

The gene NF1 encodes for the "Neurofibromin 1" protein, a negative regulator of the ras signal transduction pathway. It is a tumor suppressor gene and is inactivated either via deletions (copy number loss) or loss-of-function mutations in cancer. NF1 has been mutated in canine osteosarcoma (12.5%), glioma (16.7%), mammary cancer (0.55-14%), hemangiosarcoma (6.7-7.7%), melanoma (1.54-4.62%). NF1 loss-of-function mutations have been observed in canine melanoma (4.62%), mammary cancer (2.7-3%). NF1 loss-of-function mutations have been seen in 3.3% of human cancers, including melanoma, uterine cancer, and pheochromocytoma and paraganglioma, among others. (COSMIC, TCGA Pan-Cancer Atlas accessed via cBioPortal). NF1 loss-of-function mutations is associated with sensitivity to trametinib (a MEK inhibitor) in canine cancer cell lines.

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	NF1	Mutation	p.Arg383*

Roles in this case: Diagnostic Therapeutic

Variant Summary:

The gene NF1 encodes for the "Neurofibromin 1" protein, a negative regulator of the ras signal transduction pathway. It is a tumor suppressor gene and is inactivated either via deletions (copy number loss) or loss-of-function mutations in cancer. NF1 has been mutated in canine osteosarcoma (12.5%), glioma (16.7%), mammary cancer (0.55-14%), hemangiosarcoma (6.7-7.7%), melanoma (1.54-4.62%). NF1 loss-of-function mutations have been observed in canine melanoma (4.62%), mammary cancer (2.7-3%). NF1 loss-of-function mutations have been seen in 3.3% of human cancers, including melanoma, uterine cancer, and pheochromocytoma and paraganglioma, among others. (COSMIC, TCGA Pan-Cancer Atlas accessed via cBioPortal). NF1 loss-of-function mutations is associated with sensitivity to trametinib (a MEK inhibitor) in canine cancer cell lines.

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

Pet Name: [redacted]

Clinical Trial for this tumor type	Location	Website
AAHSD005334 - Minimally Invasive Metastasectomy in Canines (MIMIC) Trial	Veterinary Specialty Hospital - North County San Marcos, CA	Link
AAHSD005363 - Dose Escalation and Associated Toxicity Profile of Mustargen in Tumor Bearing Canine Patients	University of California-Davis Davis, CA	Link
AAHSD005435 - Preclinical Comparison of Two Hypomethylating Nucleosides in Tumor-Bearing Dogs	University of Missouri Columbia, Missouri Columbia, MO	Link
EVH Neoplasia 1 - Evaluating the Tolerability of Combination of Verdinexor and Doxorubicin in Dogs with Cancer	Ethos Veterinary Health San Rafael, CA Orland Park, IL	Link
EVH Neoplasia 2 - Evaluating the Tolerability of a Novel PCNA Inhibitor in Dogs with Cancer	Ethos Veterinary Health Woburn, MA Williston, VT	Link
KSU-VCH Neoplasia 1 - Impact of oclacitinib maleate (Apoquel?) on T regulatory cells in dogs receiving carboplatin chemotherapy for the treatment of naturally-occurring cancer	Kansas State University - Veterinary Health Center Manhattan, KS	Link
KSU-VCH Neoplasia 2 - Clinical trial for dogs with any externally accessible malignant tumor	Kansas State University - Veterinary Health Center Manhattan, KS	Link
KSU-VCH Neoplasia 3 - Effects of a new highly palatable, complete, and balanced diet for canine cancer patients	Kansas State University - Veterinary Health Center Manhattan, KS	Link
KSU-VCH Neoplasia 4 - Pain and activity monitoring in dogs receiving radiation therapy	Kansas State University - Veterinary Health Center Manhattan, KS	Link
OSU-VMC Neoplasia 1 - Lymphoma or Solid tumor-targeted chemotherapy for dogs	Ohio State University - Veterinary Medical Center Columbus, OH	Link
TU-CVMC Neoplastia - Toceranib therapy in dogs with cancer: monitoring cardiovascular toxicity, biomarkers, and assessing antihypertensive treatments	Tufts University - Cummings Veterinary Medical Center North Grafton, MA	Link
UCD-SVM Neoplasia 1 - Evaluating treatment for chemotherapy induced diarrhea in dogs receiving doxorubicin	University of California-Davis - School of Veterinary Medicine Davis, CA	Link
UCD-SVM Neoplasia 2 - Understanding more about a chemotherapy drug during a dog's cancer treatment	University of California-Davis - School of Veterinary Medicine Davis, CA	Link
UCD-SVM Neoplasia 3 - Evaluation of a single agent chemotherapy for the treatment of cancer in dogs.	University of California-Davis - School of Veterinary Medicine Davis, CA	Link
UCD-SVM Neoplasia 4 - Determining the safety and tolerability of a novel chemotherapy prodrug	University of California-Davis - School of Veterinary Medicine Davis, CA	Link
UMO-VHC Neoplasia 1 - COTC027: Preclinical Comparison of Two Hypomethylating Nucleosides in Tumor-Bearing Dogs	University Of Missouri - Veterinary Health Center Columbia, MO	Link
UWI-VC Neoplasia 1 - Evaluation of a targeted radiation	University of Wisconsin - Veterinary	Link

treatment combined with an immunotherapy treatment for dogs with metastatic cancer with an accessible tumor.	Care Madison, WI	
UWI-VC Neoplasia 2 - Dogs with histologically confirmed, high-grade lymphoma or non-sarcomatous solid tumors.	University of Wisconsin - Veterinary Care Madison, WI	Link

Other Clinical Trials That May Be Applicable

18 identified	See link for details

Variants of Uncertain Significance

The following variants were detected in Molly Covington's 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.

- BRAF (p.Thr383Ile)
- CCND1 (p.Glu316del)
- FGFR2 (p.Arg255Gln)
- KMT2D (p.Arg1403Cys)
- KMT2D (p.Arg1684Cys)
- KMT2D (p.Leu1493Phe)
- NF1 (p.Thr1218Ile)
- PTEN (p.Pro204Leu)
- TSC1 (p.Leu180Phe)

References

1. Colombo J et al. Liquid Biopsy as a Diagnostic and Prognostic Tool for Women and Female Dogs with Breast Cancer. *Cancers (Basel)* (2021). https://pubmed.ncbi.nlm.nih.gov/34680380/

2. Das S et al. Identifying Candidate Druggable Targets in Canine Cancer Cell Lines Using Whole-Exome Sequencing. *Mol Cancer Ther* (2019). https://pubmed.ncbi.nlm.nih.gov/31175136

3. Gross AM et al. Selumetinib in Children with Inoperable Plexiform Neurofibromas. *N Engl J Med* (2020). https://pubmed.ncbi.nlm.nih.gov/32187457

4. Kim TM et al. Cross-species oncogenic signatures of breast cancer in canine mammary tumors. *Nat Commun* (2020). https://pubmed.ncbi.nlm.nih.gov/32680987

5. Papalia H et al. Quick and sustained clinical response to MEK inhibitor I in a NF1 patient with neurofibromas. *Ecancermedicalscience* (2018). https://pubmed.ncbi.nlm.nih.gov/30174724

6. Py C et al. Response ofNF1-Mutated Melanoma to an MEK Inhibitor. *JCO Precis Oncol* (2018). https://ascopubs.org/doi/abs/10.1200/PO.18.00028

7. Wong K et al. Cross-species genomic landscape comparison of human mucosal melanoma with canine oral and equine melanoma. *Nat Commun* (2019). https://pubmed.ncbi.nlm.nih.gov/30664638

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

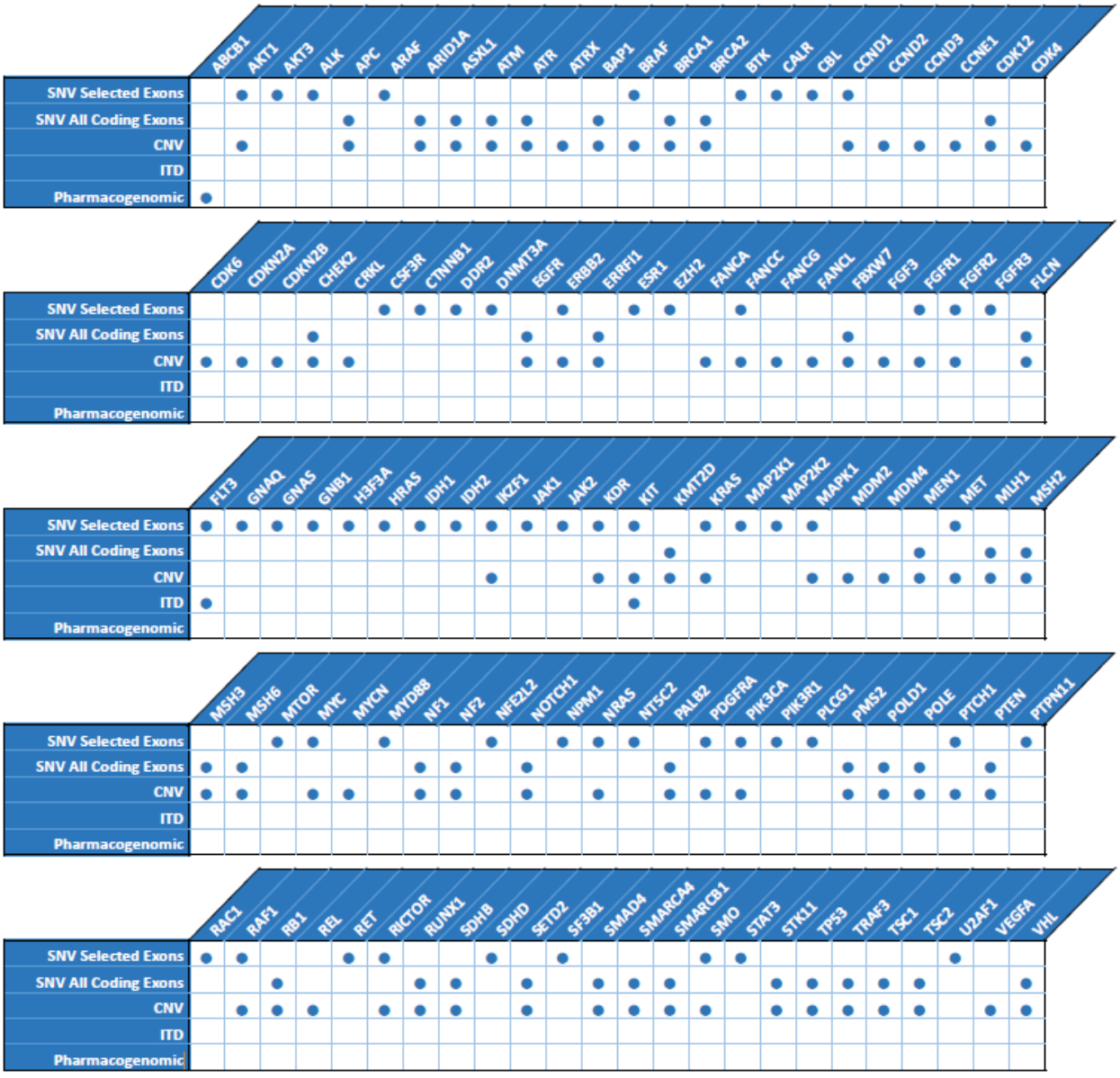
4. Mealey et al. Adverse drug reactions in veterinary patients associated with drug transporters. Vet Clin North Am Small Anim Pract (2013). https://pubmed.ncbi.nlm.nih.gov/23890239

Genes Evaluated by SearchLight DNA

Pet Name: [redacted]

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.



Assay Description

Pet Name: [redacted]

SearchLight DNA® detects multiple types of mutations in cancer genes:

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 sample. Isolated DNA is then prepared for sequencing using a custom, hybrid capture panel to enrich for target genomic regions with high actionability (Agilent). Sequencing library preparation includes shearing, purification, adaptor ligation and PCR amplification. Libraries are then clustered on a flow cell and sequenced using Illumina instruments. Sequence data are analyzed using validated bioinformatics tools (SearchLight DNA Pipeline) 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, Vidium Insight™, which contains thousands of canine cancer biomarker associations derived from primary peer-reviewed literature to identify potential pharmacogenomic, diagnostic, prognostic, and therapeutic associations. Additionally, Vidium Insight contains human cancer biomarker associations inferred via genomic and proteomic alignments and conservation scores from the Catalogue of Somatic Mutations in Cancer (COSMIC) database. 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 ABCB 1 (also known as MDR1). Indeterminate results may occur due to poor sample quality or sequencing coverage, but a "qualified report" may be delivered in these below-threshold instances after a manual data review. Mean target coverage for tumor sample DNA is ≥ 200x (unique reads) and ≥ 89% of target bases bearing ≥ 100x coverage.

Limitations

Samples with 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. Mutations 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 mutation since technical limitations in some genomic 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 mutations and are current as of the report date. Mutations may not be listed in order of strength of evidence or appropriateness for the patient's disease. When the report does identify mutations 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. Drug availability in dogs is broadly referring to their availability at a reasonable price from any of the major veterinary compounding pharmacies within the United States. Genomic mutations should be considered in the context of the patient's history, risk factors and any previous genomic testing. Variants of Uncertain Significance (VUS) may be associated with potential therapies in the future. Vidium does not update reports or send notification regarding reclassification of these mutations. 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 at vidiumah.com/terms-conditions.

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