SearchLight DNA™ Clinician Report

vidium ANIMAL HEALTH a TGen subsidiary

[redacted]

Accession: V12345

Received:08/17/21 Reported:08/25/21

Pet:	Owner:	Species:	Breed:	Sex:	Age:	Site:
[redacted]	[redacted]	Canine	Golden Retreiver	Male	11y	Left Caudal Oral

Diagnosis:

Oral sarcoma- r/o melanoma vs fibrosarcoma vs other

SearchLight DNA Overview

Biomarkers Identified: 8

ATM
CCND3
CD4K
CDKN2B
FBXW7
IKZF1
MDM2
RICTOR

Sample QC Metrics
Specimen Type: FFPE Scrolls
Tumor Content (>20%): 50%
Mean Target Coverage (>200x): 234x

Number of Clinical Trials:

- **This Cancer Type:** 2
- **General Cancer:** 15

8 Diagnostic Biomarkers

5 Prognostic Biomarkers

4 Matching Drugs: Olaparib, Abemaciclib, Palbociclib, Ribociclib

SearchLight DNA Summary

This test evaluated 120 cancer genes in the patient's 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. 8 alterations were identified of potential clinical significance for cancer diagnosis, prognosis or treatment.

Integrated review of the clinical history as well as pathology reports and genomic data for patients oral lesion supports the diagnosis of melanoma. Specifically, co-occurrence of high-level, focal CDK4 amplification (copy number gain) with high-level, focal MDM2 amplification (copy number gain), as seen in this sample, are common in human and canine melanoma. Deep CDKN2B deletion (copy number loss) also commonly occurs in canine and human melanoma in addition to various other cancer types.
In addition to the mutations and biomarker associations described in this report, additional recent studies not yet captured in our automated reports have identified associations of MDM2 copy number gain with canine oral melanoma. In Prouteau et al, Vet Comp Oncol, 2020, analysis of chromosome 10 and 30 copy number gains by quantitative PCR in 73 dogs with canine oral melanoma identified MDM2 copy number gain in 36 (49.3%) and CDK4 copy number gain in 30 (41.1%) cases. Additionally, MDM2 copy number gain has been recently described in 3 out of 10 (30%) cases of canine oral melanoma via droplet digital PCR (Prouteau et al, Sci Rep, 2021).

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Therapeutic Biomarkers

Treatment Options Based on Mutations

Drug	Mutation	Available for dogs	Used in humans
Olaparib	ATM Copy Number Loss	Yes[A]	Yes[B]
Abemaciclib	CDK4 Copy Number Gain	-----	Yes[A]
Palbociclib	CDK4 Copy Number Gain	Yes[A]	Yes[C]
Ribociclib	CDK4 Copy Number Gain	-----	Yes[C]

Drug Resistance-Associated Biomarkers

Drug	Mutation
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Pharmacogenomic Biomarkers

Gene	Mutation
ABCB1	Mutation detected

Diagnostic Biomarkers

Gene	Mutation	Described in: Canine cancer	Described in: Human cancer
ATM	Copy Number Loss	------	Yes[B]
CCND3	Copy Number Gain	Oligodendroglioma[C]	Yes[A]
CD4K	Copy Number Gain	------	Yes[B]
CDKN2B	Copy Number Loss	------	Yes[C]
FBXW7	p.Arg130*	------	Yes[D]
IKZF1	Copy Number Gain	------	Yes[A]
MDM2	Copy Number Gain	------	Yes[A]
RICTOR	Copy Number Gain	------	Yes[D]

Prognostic Biomarkers

Gene	Mutation	Negative Prognostic Factor in: Canine cancer	Negative Prognostic Factor in: Human cancer
CDK4	Copy Number Gain	-----	Yes[B]
CDKN2B	Copy Number Loss	Lymphoma[B]	-----
IKZF1	Copy Number Loss	-----	Yes[B]
MDM2	Copy Number Gain	-----	Yes[A]
RICTOR	Copy Number Gain	-----	Yes[A]

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:	CCND3	Mutation:	Copy Number Gain

Roles in this case: Diagnostic

Variant Summary:
CCND3 is mutated in human multiple myeloma, typically through translocation with IGH. It has also been shown to be focally amplified in a case of canine grade III oligodendroglioma.

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:	CDK4	Mutation:	Copy Number Gain

Roles in this case: Diagnostic Prognostic

Variant Summary:
CDK4 amplifications are common in human sarcoma (17%), glioblastoma (14%), adrenocortical carcinoma (7%), cholangiocarcinoma (6%), lung adenocarcinoma (5%), and other cancers. CDK4 is also amplified in some canine cancers including malignant melanoma, KIT-mutant mast cell tumors, and T cell lymphoma. CDK4 and CDK6 gains, like CDKN2A and RB1 loss, can lead to dysregulation of cell cycle pathways, more aggressive tumor biology, and are associated with responses to CDK4/6 inhibitors in tumors with otherwise intact RB pathways. They are also associated with poor prognosis in human liposarcoma.

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:	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:	FBXW7	Mutation:	p.Arg130*

Roles in this case: Diagnostic

Variant Summary:
FBXW7 inactivating mutations are common in human colorectal and endometrial cancers. FBXW7 mutations have also been described in canine diffuse large B-cell lymphoma and mammary 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.

Gene:	IKZF1	Mutation:	Copy Number Loss

Roles in this case: Prognostic

Variant Summary:
IKZF1 is located on chromosome 18 of the canine genome. IKZF1 is disrupted in various human cancers by mutation and, less frequently, by deletion. Disruption of IKZF1 is predicted to impair the tumor suppressive function of IKZF1.

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:	MDM2	Mutation:	Copy Number Gain

Roles in this case: Diagnostic Prognostic

Variant Summary:
MDM2 amplifications occur in human sarcomas (19%), bladder carcinomas (9%), glioblastomas (8%), adrenocortical carcinomas (7%), uterine carcinosarcomas (5%), lung adenocarcinomas (5%), esophageal cancers (5%), stomach cancers (5%), and other cancers. In human dedifferentiated liposarcoma, they are associated with reduced time to recurrence and shortened overall survival. In canine cancers, MDM2 gains or amplifications have been detected in ~24-27% of malignant melanomas and ~9% of KIT-mutant mast cell tumors. MDM2 amplifications are nearly always mutually exclusive with TP53 inactivation and with MDM4 amplification. All of these mutations typically result in TP53 dysregulation.

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:	RICTOR	Mutation:	Copy Number Gain

Roles in this case: Diagnostic Prognostic

Variant Summary:
RICTOR is located on canine chromosome 4. RICTOR is gained or amplified in various human cancers. Gain or amplification of RICTOR is associated with poor prognosis in human lung 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.

Clinical Trials Summary

Clinical Trial for this tumor type	Location	Website
AAHSD005146 - Use of papaverine to reduce low tumor oxygen in dogs with soft tissue sarcoma	The Ohio State University Columbus, OH	Link
AAHSD004796 - Contrast enhanced ultrasound in dogs with soft tissue sarcomas	Colorado State University Fort Collins, CO	Link

Other Clinical Trials that may be applicable

15 identified	See link for details

Variants of Unknown Significance

The following variants were detected in [redacted] 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.

- ARID1A(p.Ala822Thr)
- FANCG(Copy Number Loss)

References

1. Abida W et al. Genomic correlates of clinical outcome in advanced prostate cancer. *Proc Natl Acad Sci U S A* (2019). https://pubmed.ncbi.nlm.nih.gov/31061129

2. Asada H et al. Clinical significance of the two-base insertion mutation in the TP53 gene in canine histiocytic sarcoma. *Res Vet Sci* (2019). https://pubmed.ncbi.nlm.nih.gov/30852355

3. Chen Z et al. TP53 Mutations and Survival in Osteosarcoma Patients: A Meta-Analysis of Published Data. *Dis Markers* (2016). https://pubmed.ncbi.nlm.nih.gov/27239089

4. Cleary SP et al. Identification of driver genes in hepatocellular carcinoma by exome sequencing. *Hepatology* (2013). https://pubmed.ncbi.nlm.nih.gov/23728943

5. 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

6. Devillier R et al. Role of ASXL1 and TP53 mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes. *Oncotarget* (2015). https://pubmed.ncbi.nlm.nih.gov/25860933

7. Fisher OM et al. The prognostic value of TP53 mutations in oesophageal adenocarcinoma: a systematic review and meta-analysis. *Gut* (2017). https://pubmed.ncbi.nlm.nih.gov/26733670

8. Kandoth C et al. Mutational landscape and significance across 12 major cancer types. *Nature* (2013). https://pubmed.ncbi.nlm.nih.gov/24132290

9. Kirpensteijn J et al. TP53 gene mutations in canine osteosarcoma. *Vet Surg* (2008). https://pubmed.ncbi.nlm.nih.gov/18986312

10. Lorch G et al. Identification of Recurrent Activating HER2 Mutations in Primary Canine Pulmonary Adenocarcinoma. *Clin Cancer Res* (2019). https://pubmed.ncbi.nlm.nih.gov/31431454

11. McIntyre CA et al. Alterations in driver genes are predictive of survival in patients with resected pancreatic ductal adenocarcinoma. *Cancer* (2020). https://pubmed.ncbi.nlm.nih.gov/32573775

12. Parry M et al. Genetics and Prognostication in Splenic Marginal Zone Lymphoma: Revelations from Deep Sequencing. *Clin Cancer Res* (2015). https://pubmed.ncbi.nlm.nih.gov/25779943

13. Qin K et al. Prognostic value of TP53 concurrent mutations for EGFR- TKIs and ALK-TKIs based targeted therapy in advanced non-small cell lung cancer: a meta-analysis. *BMC Cancer* (2020). https://pubmed.ncbi.nlm.nih.gov/32299384

14. Rushton CK et al. Genetic and evolutionary patterns of treatment resistance in relapsed B-cell lymphoma. *Blood Adv* (2020). https://pubmed.ncbi.nlm.nih.gov/32589730

15. Stengel A et al. TP53 mutations occur in 15.7% of ALL and are associated with MYC-rearrangement, low hypodiploidy, and a poor prognosis. *Blood* (2014). https://pubmed.ncbi.nlm.nih.gov/24829203

16. Zenz T et al. TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol (2010). https://pubmed.ncbi.nlm.nih.gov/20697090

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

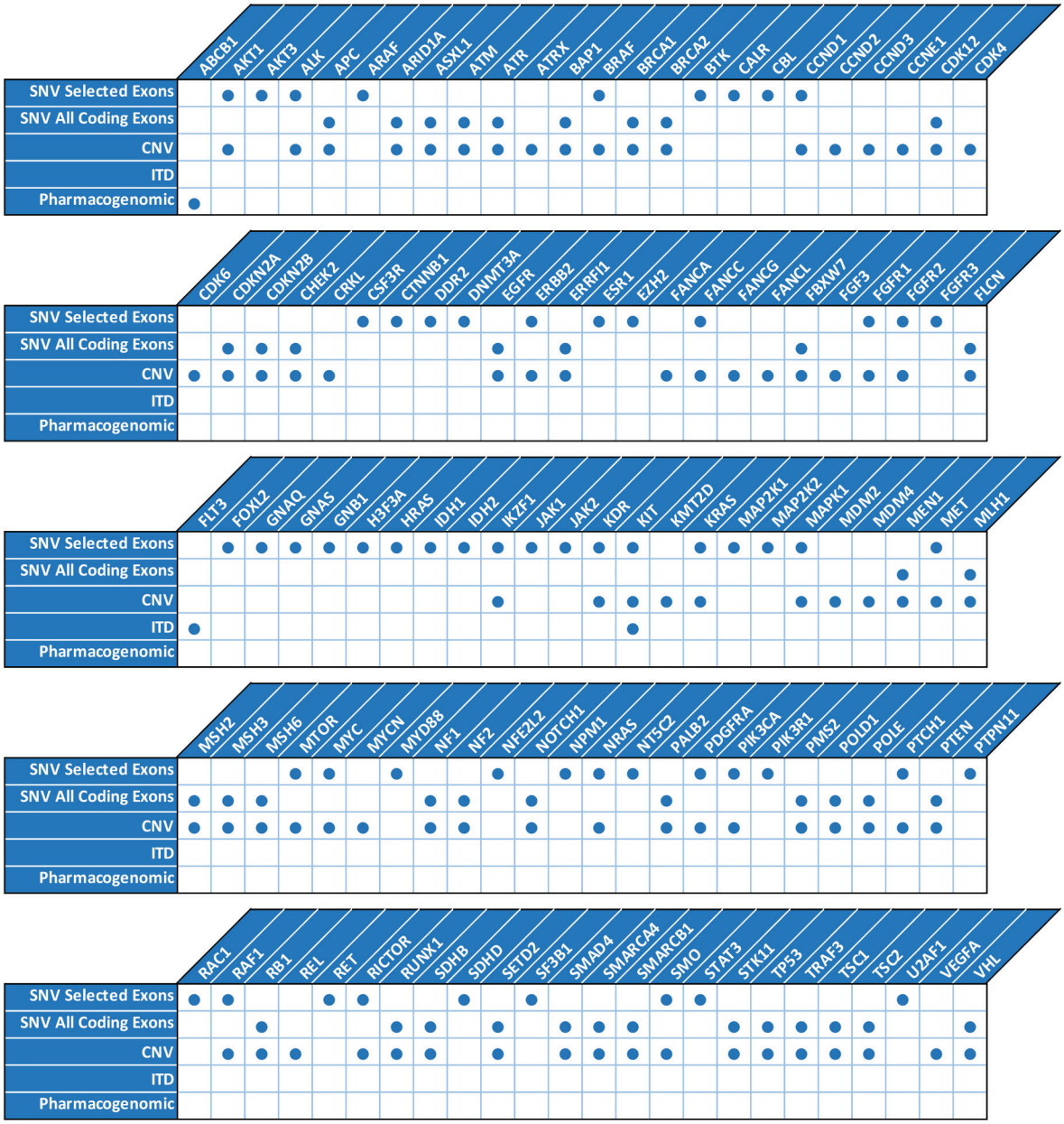
3. Mealey et al. ABCB1-1Delta polymorphism can predict hematologic toxicity in dogs treated with vincristine. J Vet Intern Med (2008). https://pubmed.ncbi.nlm.nih.gov

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

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

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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