SearchLight DNA™ Clinician Report

vidium ANIMAL HEALTH a TGen subsidiary

[redacted]

Accession: V12345

Received:10/22/21 Reported:10/29/21

Pet:	Owner:	Species:	Breed:	Sex:	Age:	Site:
[redacted]	[redacted]	Canine	Australian Shepard	Male	3y	Left proximal femur

Diagnosis:
OSA

SearchLight DNA Overview

Biomarkers Identified: 11

BAP1	**NF2**	**TP53**
BRCA2	**PTEN**	
CHEK2	**RB1**	
MEN1	**SETD2**	
MSH2	**SMARCB1**	

Sample QC Metrics
Specimen Type: FFPE Slides
Tumor Content (>20%): 90%
Mean Target Coverage (>200x): 337x

Number of Clinical Trials:

- **This Cancer Type:** 21
- **General Cancer:** 15

11 Diagnostic Biomarkers

5 Prognostic Biomarkers

5 Matching Drugs: Niraparib, Olaparib, Rucaparib, Talazoparib, Everolimus

SearchLight DNA Summary

This test evaluated 120 cancer genes in patient's tumor sample. The ABCB1-1Δ (MDR1-1Δ) mutation was detected and carries potential implications for treatment with chemotherapy. 13 alterations were identified of potential clinical significance for cancer diagnosis, prognosis or treatment.

Integrated review of patient's genomic data, clinical history, and pathology reports for this femur sample supports the diagnosis of osteosarcoma. Specifically, mutations in TP53, SETD2, PTEN and RB1 are common in canine and/or human osteosarcoma. Notably, in a recent large study of canine osteosarcoma, TP53 was the most frequently altered gene and SETD2 was the second most frequently altered gene, both of which likely play an oncogenic driver role in osteosarcoma. (Sakthikumar S, etc., Cancer Res. 2018; PMID: 29724721)

In addition to the mutations and biomarker associations described on these summary pages, additional mutations in this sample with biomarker associations not yet captured in our automated reports include copy number losses of BAP1, CHEK2 and NF2 in canine osteosarcoma. In Chu et al, Plos One, 2021, the mutational landscape of primary and pulmonary metastatic tumors in two dogs with appendicular osteosarcoma was evaluated by whole genome sequencing, whole exome sequencing and RNA sequencing. CHEK2 and NF2 copy number losses were identified in the primary tumors of both dogs. BAP1 copy number loss was also identified in the primary and metastatic tumor of one dog and the primary tumor of the other dog.

SearchLight DNA™ Clinician Report

Therapeutic Biomarkers

Treatment Options Based on Mutations

Drug	Mutation	Available for dogs	Used in humans
Niraparib	BRAC2 Copy Number Loss	------	Yes
Olaparib	BRAC2, CHEK2, FANCL Copy Number Loss	Yes	Yes
Rucaparib	FANCL Copy Number Loss	------	Yes
Talazoparib	BRAC2 Copy Number Loss	------	Yes
Everolimus	PTEN Copy Number Loss	------	Yes

Drug Resistance-Associated Biomarkers

Drug	Mutation
--	--

Pharmacogenomic Biomarkers

Gene	Mutation
ABCB1	Mutation detected

SearchLight DNA™ Clinician Report

Diagnostic Biomarkers

Gene	Mutation	Described in: Canine cancer	Described in: Human cancer
BAP1	Copy Number Loss	-----	Yes[B]
BRAC2	Copy Number Loss	Mammary Cancer[B]	Yes[A]
CHEK2	Copy Number Loss	Mammary Cancer[B]	Yes[A]
MEN1	Copy Number Loss	-----	Yes[C]
MSH2	Copy Number Loss	-----	Yes[B]
NF2	Copy Number Loss	Mammary Cancer[B]	Yes[A]
PTEN	Copy Number Loss	Osteosarcoma[A]	Yes[B]
RB1	Copy Number Loss	Osteosarcoma[B]	Yes[A]
SETD2	Copy Number Loss	Osteosarcoma[B]	Yes[D]
SMARCB1	Copy Number Loss	-----	Yes[D]
TP53	Copy Number Loss	Osteosarcoma[B]	Yes[C]

Prognostic Biomarkers

Gene	Mutation	Negative Prognostic Factor in: Canine cancer	Negative Prognostic Factor in: Human cancer
IKZF1	Copy Number Loss	------	Yes[B]
PTEN	Copy Number Loss	Mast Cell Tumor	Yes[A]
RB1	Copy Number Loss	------	Yes[B]
SETD2	Copy Number Loss	------	Yes[C]
TP53	Copy Number Loss	Mast Cell Tumor	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:	BAP1	Mutation:	Copy Number Loss

Roles in this case: Diagnostic

Variant Summary:
BAP1 is located on canine chromosome 20. BAP1 is disrupted by mutation or gene deletion in various human cancers. Disruption of BAP1 is predicted to impair the tumor suppressive function of BAP1.

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:	BRAC2	Mutation:	Copy Number Loss

Roles in this case: Diagnostic Therapeutic

Variant Summary:
The BRCA2 tumor suppressor is frequently inactivated via deletion or loss-of-function mutation in sporadic and hereditary human breast and ovarian cancers. BRCA2 deletion is predicted to disrupt the tumor suppressive function of BRCA2 and impair DNA repair processes, particularly homologous recombination repair. BRCA2 disruption is a predictive biomarker in human cancer, associated with increased sensitivity to PARP inhibition. BRCA2 copy number loss has been identified in ~2% of 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.

Gene:	CHEK2	Mutation:	Copy Number Loss

Roles in this case: Diagnosti 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.

Mutation Summaries

Gene:	FANCL	Mutation:	Copy Number Loss

Roles in this case Therapeutic

Variant Summary:
FANCL is located on canine chromosome 10. FANCL is disrupted in various human cancers by mutation and, less frequently, by deletion. Disruption of FANCL is predicted to impair the tumor suppressive function of FANCL. It has also been associated with sensitivity 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:	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.

Gene:	MEN1	Mutation:	Copy Number Loss

Roles in this case: Diagnostic

Variant Summary:
The MEN1 tumor suppressor is frequently inactivated via deletion or truncating mutation in sporadic or hereditary human parathyroid tumors and pancreatic neuroendocrine tumors. MEN1 deletions have been identified in canine prostate carcinoma and mammary carcinoma.

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:	MSH2	Mutation:	Copy Number Loss

Roles in this case: Diagnostic

Variant Summary:
MSH2 is located on canine chromosome 10. MSH2 is disrupted in various human cancers by mutation or, less frequently, by deletion. Disruption of MSH2 is predicted to impair the tumor suppressive function of MSH2. Loss of MSH2 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.

Mutation Summaries

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.

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:	RB1	Mutation:	Copy Number Loss

Roles in this case: Diagnostic Prognostic

Variant Summary:
RB1 deletions occur in a subset of sarcomas (15%), prostate cancers (9%), uterine carcinosarcomas (9%), ovarian cancers (9%), bladder cancers (9%), liver cancers (5%), and other cancers. RB1 deletion has also been associated with poor outcome in human prostate cancer. The canine chromosome 22 genomic locus encompassing the RB1 gene is frequently mutated or deleted in canine cancers including histiocytic sarcoma (~56%), malignant melanoma (~30-35%), osteosarcoma (~29%), and KIT-mutant mast cell tumors (~18%).

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:	SETD2	Mutation:	Copy Number Loss

Roles in this case: Diagnostic Prognostic

Variant Summary:
SETD2 is a tumor suppressor gene commonly inactivated through point mutations in human clear cell renal cell carcinoma and through deletion in human diffuse large B-cell lymphoma. SETD2 copy number deletions have been identified in ~21% of canine osteosarcoma.

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.

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
AAHSD000081 - Evaluating a targeted her2/neu cancer vaccine for the stimulation of anti-tumor immunity and prolonging survival times in dogs with osteosarcoma (bone cancer)	Guardian Veterinary Specialists Brewster, NY New York, NY	Link
AAHSD005054 - Safety and effectiveness of Gem-IB/docetaxel in dogs with osteosarcoma	Colorado State University Fort Collins, CO	Link
AAHSD005123 - Cryoablation with immunotherapy to treat canineosteosarcoma	Center for Image-Guided Animal Therapy at Johns Hopkins University Baltimore, MD	Link
AAHSD005139 - Analgesic effects and tolerability of tapentadol in combination with NSAIDs in dogs with appendicular osteosarcoma	The Ohio State University Columbus, OH	Link
AAHSD005141 - Canine natural killer (NK) cell therapy	The Ohio State University Columbus, OH	Link

Other Clinical Trials that may be applicable

17 identified	See link for details

Variants of Unknown Significance

The following variants were detected in Mollie Worth'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.

- KMT2D(Copy Number Loss)
- MSH6(Copy Number Loss)
- POLE(Copy Number Loss)
- RUNX1(Copy Number Gain)
- TP53(p.Arg301Gln)

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

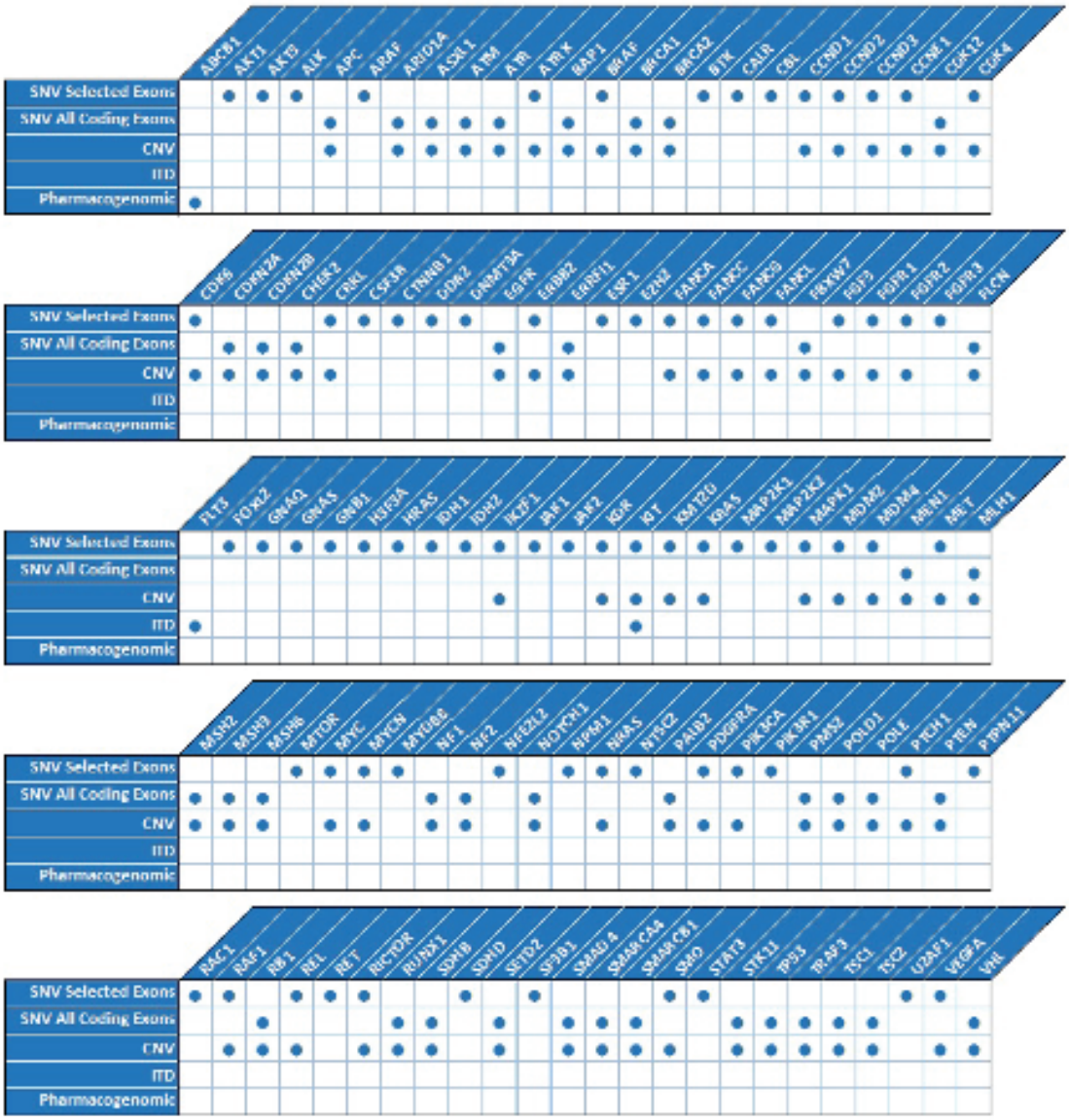
3. Mealey et al. ABCB1-1Delta polymorphism can predict hematologic toxicity in dogs treated with vincristine. J Vet Intern Med (2008). https://pubmed.ncbi.nlm.nih.gov

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 1.0) 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**.

Confidential and Information Notice

This document is CONFIDENTIAL and contains proprietary information and intellectual property of Vidium Animal Health that shall not be used, disclosed, or reproduced, in whole or in part, for any purpose, other than in accordance with the Terms and Conditions of Service or other written agreement with Vidium Animal Health. Any use, disclosure, or reproduction of any information in this document, other than for the treatment of the specific pet for which the information is intended, is strictly prohibited unless Vidium Animal Health otherwise agrees in writing. Vidium Animal Health retains title to this document and all proprietary information and intellectual property of Vidium Animal Health contained herein.

Electronically Signed by:

William Hendricks, PhD
Chief Scientific Officer