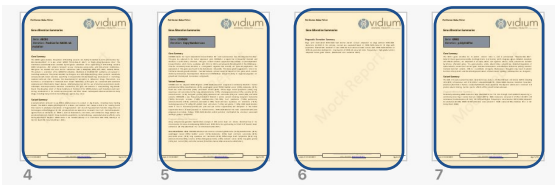
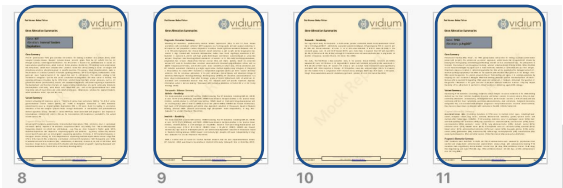
SearchLight DNA™
Orientation

The SearchLight DNA™ report is designed to describe the mutations identified in a pet's tumor. It also describes diagnostic, prognostic, and treatment-related biomarker associations with those mutations that are based on curation of more than 274 primary canine publications and inference from human cancer biomarker publications and databases. The report is thus built on a foundation of peer-reviewed research to support clinical decision-making. The front pages summarize actionable mutations and their associations. These pages are followed by in-depth biomarker summaries, evidence statements, and links to supporting articles.

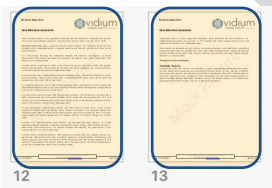
The Clinician's Report

Overview of Findings



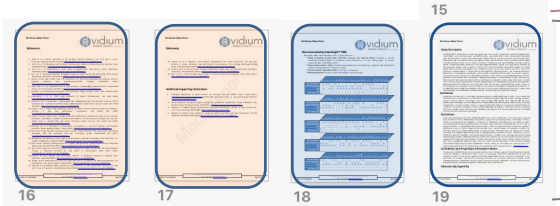


Evidence Summaries



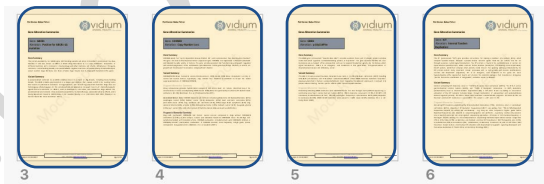
Clinical Trials

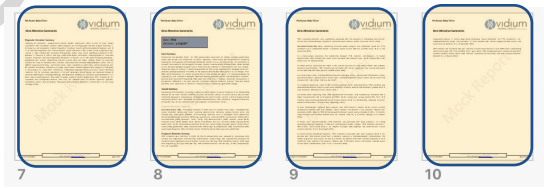
Variants of Uncertain Significance

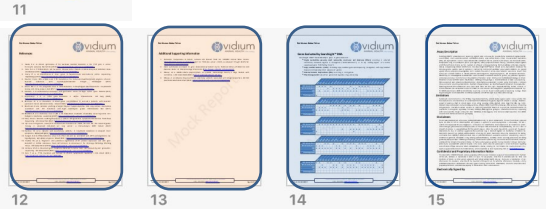


References
Gene List
Test Description

The Client's Report







SearchLight DNA™ Clinician Report

vidium ANIMAL HEALTH a TGen subsidiary

Pet Name: Boba Fisher Breed: Australian Shepherd Sex: M/N Date of Birth: August 2012 Owner: George Fisher	Veterinarian: Dr. Aphra Veterinary Clinic: Veterinary Specialty Hospitals Sample Collection Date: 2020-03-10 Sample Received Date: 2020-03-20 Report Date: 2020-03-30	**Diagnosis: Cutaneous Mast Cell Tumor** Specimen Location: Skin of Lip Specimen Type: Fine Needle Aspirate Client Accession #: 54321

Overview: This test evaluated 120 cancer genes in Boba's tumor sample. Four alterations were identified of potential clinical significance for cancer diagnosis, prognosis, or treatment.

Summary of Key Findings

Therapeutically Actionable Genomic Findings
- 1 Pharmacogenomic Biomarker
- Treatment Options Based on Genomic Alterations
  - 2 Actionable Biomarkers
  - 3 Matching Drugs

Tumor Histology-Related Findings
- 4 Diagnostic Biomarkers
- 3 Prognostic Biomarkers
- 5 Clinical Trials

Therapeutically Actionable Genomic Findings

Pharmacogenomic Biomarker

Gene	Alteration	Pharmacogenomic Association

Treatment Options Based on Genomic Alterations*

Gene	Alteration	Therapeutic Options Used or Available in Dog Oncology	Other Therapeutic Options from Human Oncology	Indication
KIT	Internal Tandem Duplication	Toceranib - -	- Axitinib Imatinib	Sensitivity Sensitivity Sensitivity
TP53	p.Arg265*	-	Trametinib	Resistance

*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.

SearchLight DNA™ Clinician Report

vidium ANIMAL HEALTH a TGen subsidiary

Pet Name: Boba Fisher
Breed: Australian Shepherd
Sex: M/N
Date of Birth: August 2012
Owner: George Fisher

Veterinarian: Dr. Aphra
Veterinary Clinic: Veterinary Specialty Hospitals
Sample Collection Date: 2020-03-10
Sample Received Date: 2020-03-20
Report Date: 2020-03-30

Diagnosis: Cutaneous Mast Cell Tumor
Specimen Location: Skin of Lip
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Tumor Histology-Related Findings

Diagnostic Biomarkers

Gene	Alteration	Diagnostic Role in Dog Cancers	Diagnostic Role in Human Cancers
CDKN2B	Copy Number Loss	✔	✔
GNB1	p.Gly116Phe	✔	-
KIT	Internal Tandem Duplication	✔	-
TP53	p.Arg265*	-	✔

Prognostic Biomarkers

Gene	Alteration	Prognostic Role in Dog Cancers	Prognostic Role in Human Cancers
CDKN2B	Copy Number Loss	Poor Outcome	-
KIT	Internal Tandem Duplication	Poor Outcome	-
TP53	p.Arg265*	Poor Outcome	Poor Outcome

Pet Name: Boba Fisher
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Diagnosis: Cutaneous Mast Cell Tumor
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Clinical Trials Summary*

Tumor Histology	Clinical Trials
Cancer	AAHSD000002 - Targeted electrochemotherapy cancer treatment for local cancer control
Cancer	AAHSD000080 - Evaluating a targeted telomerase vaccine to stimulate anti-tumor immunity and prolong survival times in dogs and cats with various cancers
Cancer	AAHSD000083 - Endoscopic/laparoscopic treatment of inoperable cancer with a targeted endoscopic electroporation system (EES)
Cancer	AAHSD004609 - Combination of electrochemotherapy and gene therapy with canine IL-12
Cancer	AAHSD004967 - Integrating immunotherapy with radiotherapy to improve tumor control for canine cancer patients

*Clinical trials are identified utilizing the American Veterinary Medical Association (AVMA) Animal Health Studies Database (AAHSD) at https://ebusiness.avma.org/aahsd/study_search.aspx based on tumor type match, then general cancer (solid tumors, hematological malignancies, neoplasia). Vidium Animal Health is not an active participant in the clinical trials shown here and has no ownership stake in any of the treatments under study.

Gene Alteration Summaries

Gene: **CDKN2B**
Alteration: **Copy Number Loss**

Gene Summary

CDKN2B stands for "cyclin dependent kinase inhibitor 2B" and is pronounced "See-Dee-Kay-Enn-Too-Bee". This gene lies adjacent to the tumor suppressor gene CDKN2A in a region that is frequently mutated and deleted in a wide variety of tumors. This gene is itself a tumor suppressor that encodes a cyclin-dependent kinase inhibitor, which forms a complex with CDK4 or CDK6, and prevents the activation of the CDK kinases. Thus the encoded protein functions as a cell growth regulator that controls cell cycle G1 progression. The expression of this gene was found to be dramatically induced by TGF beta, which suggested its role in the TGF beta induced growth inhibition. Two alternatively spliced transcript variants of this gene, which encode distinct proteins, have been reported. Mutations in CDKN2B can disrupt its ability to negatively regulate cell growth and thereby lead to excessive cell growth.

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.

Diagnostic Biomarker Summary

Array comparative genomic hybridization analysis of 109 canine mast cell tumors identified loss of the chromosome 11 locus encompassing CDKN2A and CDKN2B to be significantly enriched in KIT-mutant mast cell tumors (21.2%). (Mochizuki H et al. Chromosome Res 2017).

Associated human data. CDKN2B deletions are common in human glioblastoma (55%), mesothelioma (43%), esophageal cancer (35%), bladder cancer (31%), melanoma (29%), head and neck carcinoma (28%), pancreatic cancer (27%), lung squamous cell carcinoma (26%), diffuse large B-cell lymphoma (25%), lung adenocarcinoma (16%), sarcoma (15%), cholangiocarcinoma (14%), stomach cancer (11%), low grade glioma (11%), liver cancer (5%), and other cancers (TCGA Pan-Cancer Atlas accessed via cBioPortal).

Gene Alteration Summaries

Prognostic Biomarker Summary

Dogs with inactivated CDKN2A/B had shorter overall survival compared to dogs without CDKN2A/B alterations (p=0.02). In this analysis, survival was evaluated based on CDKN2A/B status for 40 dogs with lymphoma treated with standard of care. CDKN2A inactivation included tumors with CDKN2A/B deletion or CDKN2A promoter methylation. Inactivation of CDKN2A occurred more frequently in high-grade tumors compared to low-grade tumors. (Modiano JF et al. Leukemia 2007).

Gene Alteration Summaries

Gene: **GNB1**
Alteration: **p.Gly116Phe**

Gene Summary

The GNB1 gene encodes the G protein subunit beta 1, and is pronounced "Gee-Enn-Bee-Won". Heterotrimeric guanine nucleotide-binding proteins (G proteins), which integrate signals between receptors and effector proteins, are composed of an alpha, a beta, and a gamma subunit. These subunits are encoded by families of related genes. This gene encodes a beta subunit. Beta subunits are important regulators of alpha subunits, as well as of certain signal transduction receptors and effectors. Alternative splicing results in multiple transcript variants. GNB1 is a candidate oncogene. Mutations in this gene may result in altered function of the protein and therefore hyperactivation of downstream signaling pathways that are oncogenic.

Variant Summary

This GNB1 missense variant has been detected in one study in 12.3% of 81 mast cell tumors (MCT) including 11% (8/72) of cutaneous and 22% (2/9) of subcutaneous MCTs. These GNB1 missense mutations have been previously identified in human acute lymphoblastic B-cell leukemia. The adjacent amino acid is involved in G protein subunit binding, but the specific effects of this variant remain unknown.

Diagnostic Biomarker Summary

Potentially activating GNB1 mutations were identified for the first time through multi-platform sequencing as commonly occurring in canine mast cell tumors (MCTs). GNB1 mutations occurred in 17.3% of 81 MCTs (72 cutaneous, 9 subcutaneous; 48 fresh, 33 FFPE) with higher frequency in subcutaneous MCTs (44%) compared to cutaneous (13.9%). GNB1 G116F mutations were present in 10/81 cases (12.3%). (Vozdova M et al. Vet Comp Oncol 2020).

Gene Alteration Summaries

Gene: **KIT**
Alteration: **Internal Tandem Duplication**

Gene Summary

The KIT (pronounced "Kit") gene provides instructions for making a member of a protein family called receptor tyrosine kinases. Receptor tyrosine kinases transmit signals from the cell surface into the cell through a process called signal transduction. The KIT protein is found in the cell membrane of certain cell types where a specific protein, called stem cell factor, attaches (binds) to it. This binding turns on (activates) the KIT protein, which then activates other proteins inside the cell by adding a cluster of oxygen and phosphorus atoms (a phosphate group) at specific positions. This process, called phosphorylation, leads to the activation of a series of proteins in multiple signaling pathways. KIT is an oncogene, and mutations in this gene can cause hyperactivation of the signals that lead to cell division. The common analogy is that mutations in oncogenes "put the foot on the accelerator of cell growth" and allow cancer to develop. The signaling pathways stimulated by the KIT protein control many important cellular processes such as cell growth and division (proliferation), survival, and movement (migration). KIT protein signaling is important for the development and function of certain cell types, including reproductive cells (germ cells), early blood cells (hematopoietic stem cells), white blood cells called mast cells, cells in the gastrointestinal tract called interstitial cells of Cajal (ICCs), and cells called melanocytes. Melanocytes produce the pigment melanin, which contributes to hair, eye, and skin color.

Variant Summary

Somatic activating KIT mutations occur in ~13-50% of canine mast cell tumors (MCTs), ~35-74% of canine gastrointestinal stromal tumors (GISTs), and ~2-8% of malignant melanomas. In MCT, mutations predominantly occur as internal tandem duplications (ITD) in KIT exons 8 and 11, leading to constitutive activation of the KIT receptor tyrosine kinase through modulation of extracellular and juxtamembrane domain regulatory activity. KIT ITDs in canine MCT have been associated with prognosis and response to tyrosine kinase inhibitors. KIT exon 11 ITDs are the most common KIT mutations in canine MCTs. This variant is a KIT exon 11 ITD.

Diagnostic Biomarker Summary

Activating KIT mutations, predominantly internal tandem duplications (ITDs), commonly occur in canine mast cell tumors (MCTs). Reports of KIT mutation frequencies in MCT vary widely, from ~9% to 50%. Reported frequencies depend on cohort size and makeup - e.g. they are more frequent in higher grade MCTs. Reported frequencies also depend on sequencing regions and platforms - e.g. many studies only evaluate one or two KIT exons and use only targeted sequencing approaches. Estimates of KIT mutation frequency in the largest cohorts utilizing the most comprehensive sequencing methods report a more narrow range from 17% to 30%. Exon 11 ITDs or deletions are the most common KIT mutations in MCT followed by exon 8 ITDs or substitutions and rare mutations (ITDs, substitutions, or deletions) in exons 9, 12, and 17. KIT ITDs in MCT have been shown to drive constitutive KIT activation and downstream pro-growth signaling. (Reviewed with Consensus Guidelines in Thamm DH et al. Vet Comp Oncology 2019).

Gene Alteration Summaries

Prognostic Biomarker Summary

Activating KIT mutations, predominantly internal tandem duplications (ITDs) in exon 11, have shown associations with canine mast cell tumor (MCT) prognosis (i.e. histologic grade and post-surgical outcomes) in retrospective and prospective studies. Prospective trials have found significant association between exon 8 or 11 ITDs and progression-free interval (but not overall outcome) as well as with shorter progression-free survival in dogs treated with toceranib. Retrospective studies have found significant association of KIT mutation in exons 8, 9, and 11 with high grade (both Kiupel and Patnaik systems) based on univariate analyses. They have also found significant association of exon 11 or 12 ITDs with recurrence, metastasis, progression-free survival, disease-free interval, survival time, and death, typically based on univariate analyses. KIT exon 11 mutations have also been associated with increased cell proliferation indices such as AgNOR frequency, Ki67 index, and mitotic counts. Given variability in cohort sizes, clinical annotation, and KIT mutation assessment methods (e.g. a single region versus multiple regions versus all regions of the gene) in these studies, the status of KIT mutations as independent prognostic factors are still in need of definitive validation. Per the consensus publication of the joint Veterinary Cancer Society and American College of Veterinary Pathologists' Oncology-Pathology Working Group (OPWG), KIT "mutation presence/absence is a more objective measurement than either histologic grade or mitotic index/count, both of which can be associated with considerable observer bias. Thus, this measure could still provide important objective information useful in decision-making." (Reviewed with Consensus Guidelines in Thamm DH et al. Vet Comp Oncology 2019).

Therapeutic Evidence Summary

Axitinib – Sensitivity

This study evaluated a canine MCT-cell line, CMMC1, bearing four KIT mutations including Q255del, K415E, an exon 11 ITD (Y573_N590dup), and F507fs. CMMC1 was shown to be hypersensitive to the tyrosine kinase inhibitor, axitinib, relative to a KIT wild-type cell line, HRMC, based on multi-point drug-dose-response and cell-counting assays (IC50 of 9 nM for CMMC1 versus > 10 µM for HRMC). CMMC1 also showed constitutively high levels of KIT phosphorylation and axitinib-dose-dependent reduction of those levels based on Western blotting whereas HRMC showed constitutively high phospho-KIT levels independently of drug dose. (Takeuchi Y et al. J Vet Pharmacol Ther 2012).

Imatinib – Sensitivity

This study evaluated a canine MCT-cell line, CMMC1, bearing four KIT mutations including Q255del, K415E, an exon 11 ITD (Y573_N590dup), and F507fs. CMMC1 was shown to be hypersensitive to the tyrosine kinase inhibitor, imatinib, relative to a KIT wild-type cell line, HRMC, based on multi-point drug-dose-response and cell-counting assays (IC50 of 42.3 nM for CMMC1 versus > 10 µM for HRMC). CMMC1 also showed constitutively high levels of KIT phosphorylation and axitinib-dose-dependent reduction of those levels based on Western blotting whereas HRMC showed constitutively high phospho-KIT levels independently of drug dose. (Takeuchi Y et al. J Vet Pharmacol Ther 2012).

CoMS is a canine mast cell tumor cell line that has been shown to bear the exon 11 juxtamembrane domain KIT mutation. CoMS was shown to be sensitive to imatinib in this study. (Kobayashi M et al. Oncol Rep 2017).

Gene Alteration Summaries

Toceranib – Sensitivity

This registration study for toceranib - a multi-center, placebo-controlled, double-blind, randomized clinical trial in 153 dogs with MCT - additionally evaluated association between KIT genotype by PCR for exon 11 and 12 ITDs and clinical outcomes. KIT exon 11 or 12 ITDs were detected in 20% of cases (30/150). In the toceranib group, exon 11 and 12 KIT-mutant MCTs were more likely to respond than KIT wild-type MCTs (60.0%, 12/20 versus 31.3%, 20/64), although KIT mutations were not associated with time to progression or duration of response. (London CA et al. Clin Cancer Res 2009).

This study, the first Phase I dose-escalation study of the tyrosine kinase inhibitor, toceranib (SU11654) evaluated KIT exon 11 ITD status in 22 dogs with MCT treated with SU11654. KIT exon 11 ITDs were found in 50% (11/22) of MCT cases. KIT exon 11 ITD status as well as lymph node involvement were significantly associated with initial response to therapy. KIT ITD-positive cases also survived longer (36.9 v 15.4 weeks) and the median time to progression in KIT-mutant versus wild-type MCTs was 21.0 weeks v 3.9 weeks, though these associations were not statistically significant. (London CA et al. Clin Cancer Res 2003).

Gene Alteration Summaries

Gene: **TP53**
Alteration: **p.Arg265***

Gene Summary

Pronounced "Tee-Pee-Fifty-Three", the TP53 gene provides instructions for making a protein called tumor protein p53 (or p53). This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing (proliferating) too fast or in an uncontrolled way. The p53 protein is located in the nucleus of cells throughout the body, where it attaches (binds) directly to DNA. When the DNA in a cell becomes damaged by agents such as toxic chemicals, radiation, or ultraviolet (UV) rays from sunlight, this protein plays a critical role in determining whether the DNA will be repaired or the damaged cell will self-destruct (undergo apoptosis). If the DNA can be repaired, p53 activates other genes to fix the damage. If the DNA cannot be repaired, this protein prevents the cell from dividing and signals it to undergo apoptosis. By stopping cells with mutated or damaged DNA from dividing, p53 helps prevent the development of tumors. Because p53 is essential for regulating DNA repair and cell division, it has been nicknamed the "guardian of the genome." Mutations in this gene are some of the most common mutations in cancer. They may impact the ability of the p53 protein to perform its critical functions in protecting against DNA damage.

Variant Summary

Inactivating TP53 mutations (truncating mutations and/or hotspot missense mutations in the DNA-binding domain) are the most common mutations in canine and human cancers. In canine cancers, they are most commonly observed in osteosarcoma, hemangiosarcoma, and histiocytic sarcoma (>40% of cases) and less commonly (<20%) in B-cell lymphoma, pulmonary adenocarcinoma, mast cell tumors, malignant melanoma, and glioma. They are also associated with poor prognosis in many human cancers. In canine cancer cell lines, they have also been shown to correlate with resistance to the MEK inhibitor trametinib.

Diagnostic Biomarker Summary

Associated human data. Truncating mutations in TP53 occur in multiple tumor types, including breast cancer, colorectal cancer, lung cancer, sarcoma, adrenocortical carcinoma, glioma, Spitzoid tumor, and multiple other tumor types (COSMIC). TP53 truncating mutations occur in esophageal cancer (34%), head and neck squamous cell carcinoma (32%), lung squamous cell carcinoma (30%), ovarian cancer (27%), uterine carcinosarcoma (23%), pancreatic cancer (22%), lung adenocarcinoma (20%), stomach cancer (18%), colorectal cancer (17%), bladder cancer (16%), chromophobe renal cell carcinoma (15%), sarcomas (13%), breast cancer (12%), adrenocortical carcinoma (12%), liver cancer (12%), low grade gliomas (11%), uterine cancer (10%), glioblastoma (8%), melanoma (7%), diffuse large B-cell lymphoma (5%), mesothelioma (5%), acute myeloid leukemia (5%), and other cancers. (TCGA Pan-Cancer Atlas accessed via cBioPortal).

Prognostic Biomarker Summary

TP53 mutations were identified in 24/59 (40.7%) of osteosarcoma cases assessed by polymerase chain reaction and single-strand conformational polymorphism analysis. Dogs with osteosarcoma bearing TP53 mutations had a significantly shorter median survival time (81 days, 95% confidence interval: 73-89 days) than dogs bearing wild-type TP53 (256 days, 95% confidence interval: 119-392 days, p=.026). (Kirpensteijn J et al. Vet Surg 2008).

Gene Alteration Summaries

TP53 truncating mutations were significantly associated with the presence of metastases, but not with survival time or chemotherapy response in canine histiocytic sarcoma. (Asada H et al. Res Vet Sci 2019).

Associated human data. Deep sequencing of human splenic marginal zone lymphoma found that TP53 mutations are an independent marker of reduced overall survival (HR 2.36, p=0.03). (Parry M et al. Clin Cancer Res 2015).

In a meta-analysis evaluating the relationship between TP53 mutation and prognosis in human osteosarcoma, TP53 mutation was found to be associated with reduced 2-year overall survival (RR=1.79). (Chen Z et al. Dis Markers 2016).

In a meta-analysis of patients with EGFR or ALK mutant non-small cell lung cancer treated with targeted tyrosine kinase inhibitors, TP53 mutation was associated with shorter progression free survival and overall survival (HR=1.88, HR=1.92). (Qin K et al. BMC Cancer 2020).

In a meta-analysis study including 888 patients with esophageal cancer, patients with TP53 mutant tumors were found to have reduced overall survival when compared to patients whose tumors did not have TP53 mutations (HR 1.48). (Fisher OM et al. Gut 2017).

In a targeted sequencing study of 283 primary pancreatic ductal adenocarcinoma, TP53 mutation was associated with reduced overall survival when compared to tumors without TP53 mutations (median OS 37.4 vs 65.0 months). (McIntyre CA et al. Cancer 2020).

In a whole exome sequencing study of 87 hepatocellular carcinomas, TP53 mutation was associated with a higher recurrence rate (89 vs 40 percent, p=0.006), shorter disease-free survival (median DFS 7.9 vs 42.9 months), and a trend toward reduced overall survival (median OS 26.0 vs 83.2 months), compared to tumors without TP53 mutations. (Cleary SP et al. Hepatology 2013).

In acute lymphoblastic leukemia (ALL), patients with TP53 mutation showed shorter overall survival, compared to patients with TP53 wildtype tumors (median 11.5 months vs not reached). Patients with alterations in both alleles of TP53 (2 hits) showed the shortest overall survival (median OS 2 hits, 7.1 months, single mutation, 63.5 months, deletion alone, not reached, TP53 wt, 75.5 months). (Stengel A et al. Blood 2014).

In human acute myeloid leukemia, TP53 mutations are associated with worse prognosis. In a study evaluating prognostic subgroups of AML with myelodysplasia-related changes, TP53 mutations occurred in 22% of cases, were mostly found in the complex karyotype AML subgroup, and predicted poor clinical outcome. (Devillier R et al. Oncotarget 2015).

In human chronic lymphocytic leukemia, TP53 mutation is associated with poor prognosis. None of the patients with TP53-mutant tumors had a complete response to fludarabine-based chemotherapy. The median progression-free survival for was 23.3 months for patients with TP53 mutation compared to 62.2 months for those without TP53 mutation. Patients with TP53 mutant tumors also showed reduced overall survival (29.2 vs 84.6 months). (Zenz T et al. J Clin Oncol 2010).

Gene Alteration Summaries

Longitudinal analysis of diffuse large B-cell lymphomas found enrichment for TP53 mutations in the relapsed/refractory setting, and association of TP53 mutation with inferior progression-free survival and overall survival. (Rushton CK et al. Blood Adv 2020).

TP53 mutation was associated with poor outcome in a pan-cancer analysis of over 3000 tumors representing twelve tumor types (HR 1.19, p=0.049). Tumor types where TP53 showed significant associations with poor outcome included kidney cancer, head and neck squamous cell carcinoma, and acute myeloid leukemia. (Kandoth C et al. Nature 2013).

Therapeutic Evidence Summary

Trametinib - Resistance
In a preclinical study, drug sensitivity was evaluated in a panel of genomically characterized canine cancer cell lines. Whole exome sequencing was performed for 33 canine cancer cell lines, spanning 10 different tumor types. TP53 mutations were detected in 36% (12/33) of the cell lines. Seven of the 9 osteosarcoma cell lines were trametinib resistant, including the three osteosarcoma cell lines with activating mutations in genes in the MAPK pathway: BRAF, NRAS, and KRAS. Across the full panel of cell lines, TP53 mutations were associated with trametinib resistance. (Das S et al. Mol Cancer Ther 2019).

Clinical Trials

Clinical Trial Options Based on Tumor Histology

Clinical trials are being conducted for dogs with cancer. Trials in the United States for dogs with mast cell tumor or for dogs with cancer in general are listed below and are based on investigator self-curated deposition into the AVMA AAHSD (https://ebusiness.avma.org/aahsd/study_search.aspx). Additional enrollment eligibility criteria may apply or additional trials may be available.

Tumor Histology	Clinical Trials	Location
Cancer	AAHSD000002 - Targeted electrochemotherapy cancer treatment for local cancer control	Guardian Veterinary Specialists Brewster, New York; New York, New York
Cancer	AAHSD000080 - Evaluating a targeted telomerase vaccine to stimulate anti-tumor immunity and prolong survival times in dogs and cats with various cancers	Guardian Veterinary Specialists Brewster, New York; New York, New York
Cancer	AAHSD000083 - Endoscopic/laparoscopic treatment of inoperable cancer with a targeted endoscopic electroporation system (EES)	Guardian Veterinary Specialists Brewster, New York; New York, New York
Cancer	AAHSD004609 - Combination of electrochemotherapy and gene therapy with canine IL-12	Veterinary Oncology Services New York, New York
Cancer	AAHSD004967 - Integrating immunotherapy with radiotherapy to improve tumor control for canine cancer patients	Colorado State University Fort Collins, Colorado

Appendix

Variants of Uncertain Significance

The following variants were detected in Boba'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 these mutations in cancer.

ATR (Copy Number Loss)

ATR is the gene that encodes the ATR serine/threonine kinase, and is pronounced "Ay-Tee-Ahr". The protein encoded by this gene is a serine/threonine kinase and DNA damage sensor, activating cell cycle checkpoint signaling upon DNA stress. The encoded protein can phosphorylate and activate several proteins involved in the inhibition of DNA replication and mitosis, and can promote DNA repair, recombination, and apoptosis. This protein is also important for fragile site stability and centrosome duplication. Defects in this gene are a cause of Seckel syndrome 1. Other mutations in this gene may limit the resulting protein's ability to detect and repair damaged DNA and tumorigenesis may result.

EGFR (p.Ile287Val)

The EGFR gene provides instructions for making a receptor protein called the epidermal growth factor receptor, and is pronounced "Ee-Jee-Eff-Ahr". The receptor for epidermal growth factor spans the cell membrane so that one end of the protein remains inside the cell and the other end projects from the outer surface of the cell. This positioning allows the receptor to attach (bind) to other proteins, called ligands, outside the cell and to receive signals that help the cell respond to its environment. Ligands and receptors fit together like keys into locks. Epidermal growth factor receptor binds to at least seven different ligands. The binding of a ligand to epidermal growth factor receptor allows the receptor to attach to another nearby epidermal growth factor receptor protein (dimerize), turning on (activating) the receptor complex. As a result, signaling pathways within the cell are triggered that promote cell growth and division (proliferation) and cell survival. EGFR is an oncogene. Mutations in the gene that make this protein less likely to turn off or more likely to activate can cause proliferation and tumorigenesis.

References

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Additional Supporting Information

1. Alteration frequencies in human cancers are derived from the COSMIC Cancer Gene Census (https://cancer.sanger.ac.uk/census) 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)
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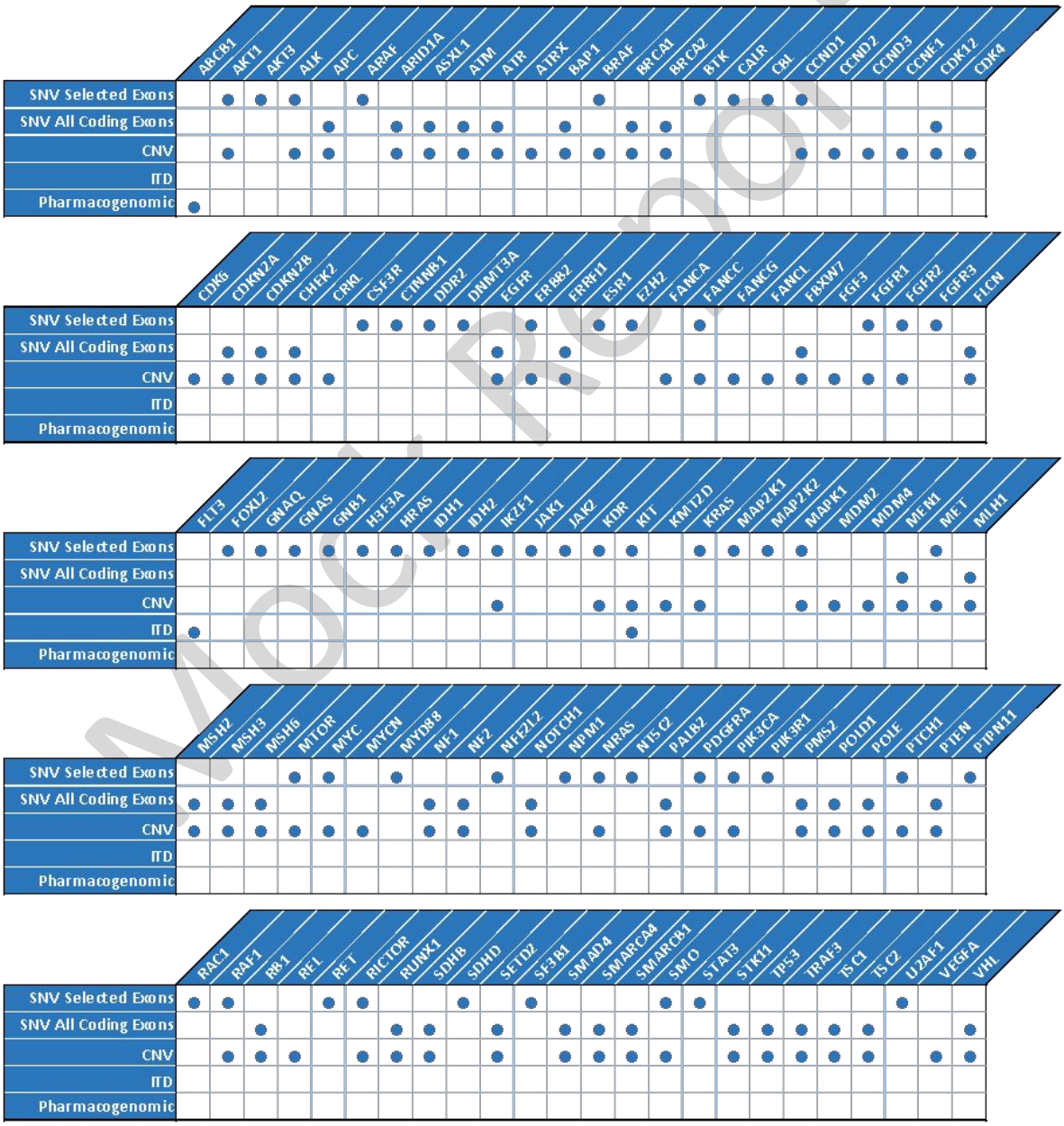
Genes Evaluated by SearchLight™ DNA

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.



Assay Description

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.

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