SearchLight DNA™ Clinician Report

[redacted]

Accession: V12345

Received:10/05/20 Reported:10/20/21

Pet:	Owner:	Species:	Breed:	Sex:	Age:	Site:
[redacted]	[redacted]	Canine	Chihuahua Mix	Male	6y	Peripheral Lymph Node

Diagnosis:
Lymphoma

SearchLight DNA Overview

Biomarkers Identified: 12

ARID1A	**MYC**	**STK11**
BAP1	**PDGFRA**	**TRAF3**
BRCA1	**RB1**	
KDR	**SDHB**	
KIT	**SETD2**	

Sample QC Metrics
Specimen Type: Fine Needle Aspirate
Tumor Content (>20%): 75%
Mean Target Coverage (>200x): 337x

Number of Clinical Trials:

- **This Cancer Type:** 4
- **General Cancer:** 3

12 Diagnostic Biomarkers

4 Prognostic Biomarkers

4 Matching Drugs: Niraparib, Olaparib, Rucaparib, Talazoparib

SearchLight DNA Summary

This test evaluated 120 cancer genes in the 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.

Most notable findings are the copy number losses impacting BRCA1 and CDK12 that are associated with PARP inhibitor responses in human cancers, though I know these aren't readily available drugs.

We also see TRAF3 mutations which are diagnostic for DLBCL among lymphomas. Though not on the report, there are hypothetical associations between TRAF3 mutation and bortezomib response in other B-cell malignancies (such as multiple myeloma).

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Therapeutic Biomarkers

Treatment Options Based on Mutations

Drug	Mutation	Available for dogs	Used in humans
Niraparib	BRAC1 Copy Number Loss	------	Yes[A]
Olaparib	BRAC1, CHEK2, FANCL Copy Number Loss	Yes[B]	Yes[C]
Rucaparib	FANCL Copy Number Loss	------	Yes[D]
Talazoparib	BRAC1 Copy Number Loss	------	Yes[B]

Drug Resistance-Associated Biomarkers

Drug	Mutation
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Pharmacogenomic Biomarkers

Gene	Mutation
ABCB1	No Mutation

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Diagnostic Biomarkers

Gene	Mutation	Described in: Canine cancer	Described in: Human cancer
ARID1A	Copy Number Loss	-----	Yes[B]
BAP1	Copy Number Loss	-----	Yes[A]
BRCA1	Copy Number Loss	-----	Yes[A]
KDR	Copy Number Loss	Lymphoma[C], Hemangiosarcoma[D]	Yes[C]
KIT	Copy Number Loss	Lymphoma[A], Hemangiosarcoma[C], Mast Cell Tumor[A], Melanoma[C], Lung Cancer[D]	Yes[B]
MYC	Copy Number Loss	Lymphoma[B]	Yes[A]
PDGFRA	Copy Number Loss	Lymphoma[B], Hemangiosarcoma[B], Glioma[C], Osteosarcoma[A,]	Yes[B]
RB1	Copy Number Loss	Osteosarcoma[B]	Yes[A]
SDHB	Copy Number Loss	Mammary Cancer[C]	Yes[D]
SETD2	Copy Number Loss	Osteosarcoma[B]	Yes[D]
STK11	Copy Number Loss	-----	Yes[C]
TRAF3	p.His179fs	Lymphoma[A]	Yes[B]
TRAF3	p.Asp324fs	Lymphoma[B]	Yes[D]

Prognostic Biomarkers

Gene	Mutation	Negative Prognostic Factor in: Canine cancer	Negative Prognostic Factor in: Human cancer
CDK12	Copy Number Loss	------	Yes[B]
MYC	Copy Number Gain	------	Yes[A]
PDGFRA	Copy Number Gain	------	Yes[B]
SETD2	Copy Number Loss	------	Yes[C]

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:	ARID1A	Mutation:	Copy Number Loss

Roles in this case: Diagnostic

Variant Summary:
ARID1A is a tumor suppressor gene that is deleted in a variety of human tumors including clear cell ovarian carcinoma, renal cell carcinoma, and breast tumors. ARID1A deletions in canine cancers have not been well-described. ARID1A deletions lead to loss of ARID1A's normal functions in chromatin regulation and transcriptional control and thereby may be associated with oncogenic processes and epigenetic dysregulation. Emerging data in human oncology also suggest tumors with ARID1A deficiency may show impaired DNA mismatch repair.

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:	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:	BRAC1	Mutation:	Copy Number Loss

Roles in this case: Diagnostic Therapeutic

Variant Summary:
The BRCA1 tumor suppressor is frequently inactivated via deletion or truncating mutation in sporadic and hereditary human breast and ovarian cancers. Somatic BRCA1 deletion and mutation have also been identified in canine mammary cancer as have candidate predisposing pathogenic BRCA1 SNPs. BRCA1 deletions have specifically been identified in canine mammary and prostate carcinoma. BRCA1 deletion is predicted to disrupt its tumor suppressive function of BRCA1 and impair DNA repair processes, particularly homologous recombination repair. BRCA1 disruption is a predictive biomarker in human cancer, associated with increased sensitivity to PARP inhibition.

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:	CDK12	Mutation:	Copy Number Loss

Roles in this case Prognostic Therapeutic

Variant Summary:
CDK12 is located on canine chromosome 9. CDK12 is disrupted in a subset of human cancers, typically by truncating mutations and less frequently due to copy number loss. Deletion of CDK12 is predicted to result in impaired or loss of CDK12 function, impairment of homologous recombination repair, and sensitivity to PARP inhibition. Deleterious mutations in CDK12 are considered a predictive biomarker for 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:	KDR	Mutation:	Copy Number Gain

Roles in this case: Prognostic

Variant Summary:
KDR is located on canine chromosome 13. KDR, also known as VEGFR2, is frequently gained or amplified alongside its upstream neighbor, KIT, in canine and human cancers. KDR is commonly amplified in canine B-cell lymphoma (~58%), hemangiosarcoma (~22-28%), pulmonary adenocarcinoma (~20%), and mammary carcinoma (>20%).

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:	KIT	Mutation:	Copy Number Gain

Roles in this case: Diagnostic

Variant Summary:
KIT is located on canine chromosome 13. KIT amplifications occur in a subset of human cancers, including glioblastoma (10%), lung squamous cell carcinoma (5%), and other cancers. KIT copy number gain or amplification also occur commonly in canine cancers such as B- and T-cell lymphoma, hemangiosarcoma, lung cancer, mast cell tumors, melanoma, 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.

Mutation Summaries

Gene:	MYC	Mutation:	Copy Number Gain

Roles in this case: Diagnostic Prognostic

Variant Summary:
MYC amplifications are common in ovarian cancer (33%), esophageal cancer (22%), uterine carcinosarcoma (21%), breast carcinoma (15%), pancreatic cancer (13%), stomach cancer (12%), liver cancer (11%), head and neck cancer (9%), uterine cancer (9%), prostate cancer (8%), lung adenocarcinoma (8%), lung squamous cell carcinoma (8%), bladder cancer (6%), colorectal cancer (5%), and other cancers. MYC is also frequently gained or amplified in many canine cancers including B-cell lymphoma (39-75%) and T-cell lymphoma (~30%), glioma (~14%), head and neck squamous cell carcinoma (~58%), hemangiosarcoma (~9%), mammary carcinoma (~25-30%), oral malignant melanoma (~80%), and osteosarcoma (~33%). In many human cancers, MYC gain or amplification is also associated with poor prognosis.

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:	PDGFRA	Mutation:	Copy Number Gain

Roles in this case: Diagnostic Prognostic

Variant Summary:
PDGFRA amplifications occur in a subset of human cancers, including glioblastoma (13%), and other cancers. PDGFRA is also frequently gained or amplified in canine B-cell lymphoma (~58%), osteosarcoma (~21%), hemangiosarcoma (~20%), and glioma (~4%). It has also been associated with human glioma tumor grade and progression-free survival.

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:	SDHB	Mutation:	Copy Number Loss

Roles in this case: Diagnostic

Variant Summary:
The SDHB gene is located on canine chromosome 2. SDHB is a tumor suppressor commonly inactivated through point mutations in paraganglioma and pheochromocytoma and it plays a role in familial paraganglioma. SDHB deletion has been identified in canine mammary cancer. SDHB is also disrupted in a subset of human cancers, typically by copy number loss or mutation. Deletion of SDHB is predicted to result in impairment or loss of SDHB function and altered cellular metabolism.

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:	STK11	Mutation:	Copy Number Loss

Roles in this case: Diagnostic

Variant Summary:
STK11 is commonly mutated in human lung and pancreatic cancer, typically through deletions and inactivating point mutations. It also plays a role in Peutz-Jeghers syndrome and is involved in germline predisposition to multiple cancer types. STK11 has also 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:	TRAF3	Mutation:	p.His179fs

Roles in this case: Diagnostic

Variant Summary:
TRAF3 is located on chromosome 8 of the canine genome. TRAF3 is disrupted in a subset of human cancers by mutation or deletion. Truncating frameshift and nonsense mutations in TRAF3 are characterized as loss-of-function mutations, and are predicted to impair the tumor suppressive function of TRAF3. TRAF3 is also commonly inactivated in canine lymphoma, typically through loss-of-function point mutations.

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:	TRAF3	Mutation:	p.Asp324fs

Roles in this case: Diagnostic

Variant Summary:
TRAF3 stands for "TNF receptor associated factor 3" and is pronounced "Traff-Three". The protein encoded by this gene is a member of the TNF receptor associated factor (TRAF) protein family. TRAF proteins associate with, and mediate the signal transduction from, members of the TNF receptor (TNFR) superfamily. This protein participates in the signal transduction of CD40, a TNFR family member important for the activation of the immune response. This protein is found to be a critical component of the lymphotoxin-beta receptor (LTbetaR) signaling complex, which induces NF-kappaB activation and cell death initiated by LTbeta ligation. Epstein-Barr virus encoded latent infection membrane protein-1 (LMP1) can interact with this and several other members of the TRAF family, which may be essential for the oncogenic effects of LMP1. TRAF3 is a tumor suppressor gene for which mutations may disrupt the ability of the TRAF3 protein to perform its critical functions.

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
AAHSD005097 - Efficacy of Rabacfosadine, Vincristine, Cyclophosphamide, Doxorubicin, and Prednisone (T-CHOP) in Dogs with Untreated Lymphoma	Oregon State University Corvallis, Oregon	Link
AAHSD004852 - Open-label, phase-2 trial of Immunolight therapy for dogs with Non-Hodgkin's Lymphoma	North Carolina State University Raleigh, North Carolina	Link
AAHSD004968 - Phase I/II study of temozolomide in cancer bearing dogs	Auburn University Auburn, Alabama	Link
AAHSD004555 - CHOP Dose Escalation for Canine Lymphoma	The Ohio State University Columbus, OH	Link

Other Clinical Trials that may be applicable

3 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.

- FGFR2 (p.Thre528Met)
- PMS2 (p.Asp552_Ala553del)
- MSH2 (p.Met210Val)
- POLD1 (p.Ala895Val)
- SMARCA4 (Copy Number Loss)
- TRAF3 (p.Leu498Pro)
- TSC2 (p.Val645Ile)

References

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Additional Supporting Information

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

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

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

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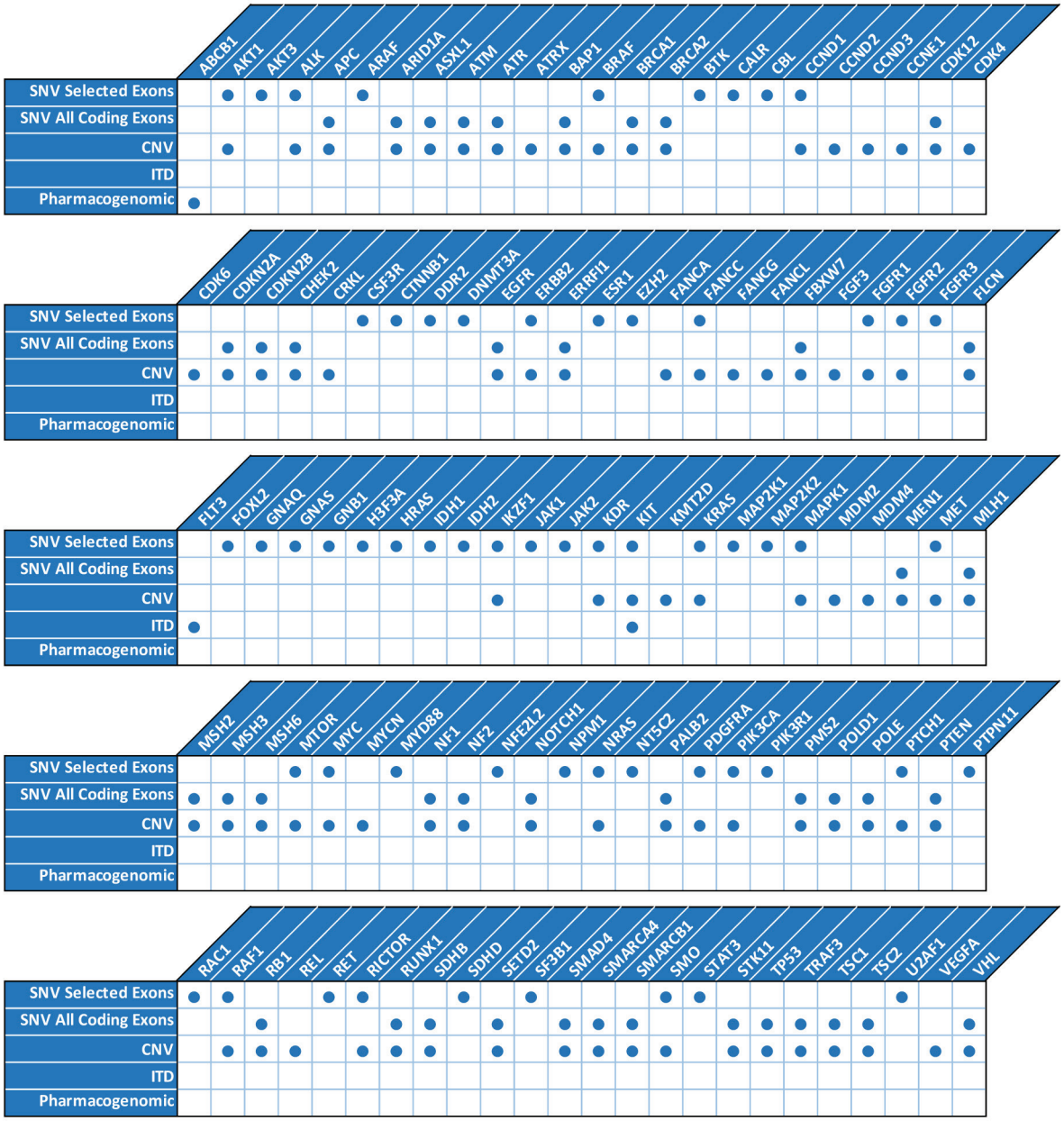
Genes Evaluated by SearchLight™ DNA

SearchLight DNA™ detects multiple types of gene mutations:

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



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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