ONCOLOGY genpath an OPKO Health Company

OnkoSight™ Advanced Chronic Lymphoid Neoplasm NGS Report

Final Report

SAMPLE, PHYSICIAN
Oncology Hospital
100 Main Street
Anytown, USA 00000

Patient Name: SAMPLE, PATIENT
DOB: **Age:** **Sex:**
Surgical #:
Patient ID:

Specimen ID: XXXXXXXXX
Date of Report: 06/20/2023 01:54 PM EDT
Date Collected: 06/13/2023
Date Received: 06/14/2023
Specimen Source: Bone Marrow

DETECTED STRUCTURAL ALTERATIONS: Trisomy 12 (+12), Chromosome 13q loss involving RB1 region, and deletion of TP53 (17p13) locus were detected.
See section "Genome-Wide Distribution of CNV and SNV" for details.

RESULT SUMMARY: ABNORMAL

DETECTED GENOMIC ALTERATIONS:

Tier I: Variants of Strong Clinical Significance

NOTCH1 p.(Pro2514ArgfsTer4)

TP53 p.(Arg181Gly)

TP53 p.(Val173Leu)

TUMOR TYPE:

Suspected Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

CLINICAL INFORMATION:

The diagnosis under consideration is CLL/SLL.

IMMUNOTHERAPY BIOMARKERS:

TUMOR MUTATION BURDEN: LOW (1.6 MUTATIONS / MB)

MICROSATELLITE INSTABILITY: MSI NEGATIVE (1.64%)

PERTINENT NEGATIVE RESULTS

The following genes are NEGATIVE for clinically relevant mutations. Mutational hotspots and surrounding exonic regions were interrogated for DNA level point mutations and indels (fusions not assayed).

ATM, BCL2, BCL6, BIRC3, BRAF, BTK, CARD11, CD79B, CDKN2A, CREBBP, CXCR4, DNMT3A, EP300, EZH2, IDH1, IDH2, IKZF1, IRF4, JAK1, JAK3, KMT2D, KRAS, MAP2K1, MEF2B, MYC, MYD88, NOTCH2, NRAS, PIK3CA, PIK3CG, PLCG2, PTPRD, RHOA, SETD2, SF3B1, STAT3, STAT5B, TET2, TNFRSF14

TECHNICAL SUMMARY

Gene	Alteration	AMP Tier	Chr	Pos	Ref	Alt	Coverage	Allele Freq. or Fold Change	cDNA Change	Exon
NOTCH1	p.(Pro2514ArgfsTer4)	I	9	139390648	AG	-	2379	38%	c.7541_7542 del	34
TP53	p.(Arg181Gly)	I	17	7578389	G	C	1457	49%	c.541C>G	5
TP53	p.(Val173Leu)	I	17	7578413	C	A	1556	9%	c.517G>T	5

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Patient Name: SAMPLE, PATIENT DOB: **Specimen ID: XXXXXXXXX**

PROGNOSTIC ASSOCIATIONS

	Gene	Alteration	Prognostic Association	Disease Association
↓	TP53	p.(Arg181Gly)	TP53 mutated CLL/SLL is associated with high risk disease	CLL/SLL

THERAPEUTIC ASSOCIATIONS

In Patient's Tumor Type

	Gene / Locus	Alteration	Potential Therapeutic Response / Drug Class	Disease Association
✓	TP53	p.(Arg181Gly)	Targeted therapy agents ibrutinib, idelalisib, venetoclax, obinutuzumab	CLL/SLL

INTERPRETATION SUMMARY

DETECTED STRUCTURAL ALTERATIONS: Trisomy 12 (+12), Chromosome 13q loss involving RB1 region, and deletion of TP53 (17p13) locus were detected.

See section "Genome-Wide Distribution of CNV and SNV" for details.

Two mutations in TP53 (p.(Arg181Gly) & p.(Val173Leu)) were detected in this patient's sample.

Manual inspection of NGS read data confirms the detected TP53 mutations exhibit trans configuration (i.e. involving different alleles), compatible with clonal heterogeneity. Next generation sequencing allows for the resolution of single molecule mutation status and review of the data revealed that these mutations were not found on the same DNA molecule (not in cis) and therefore affecting different copies of the gene. This means that these alterations are either in trans (different DNA molecules) in the same clone, or found in different, distinct clones.

TP53 mutations are known to be nonspecifically recurrent across the spectrum of hematopoietic malignancies, including lymphoplasmacytic lymphomas (WHO Haematopoietic and Lymphoid Tissues, 2023). Therapeutically predictive or prognostic implications for this genomic alteration are not well established or formally incorporated into clinical algorithms for LPL (NCCN Guidelines, Waldenström Macroglobulinemia/Lymphoplasmacytic Lymphoma, Version 1.2023).

TP53 mutations are also indicators of higher risk disease stratification in CLL/SLL. TP53 mutation is associated with especially poor prognosis when identified in conjunction with unmutated IGHV somatic hypermutation status. In addition, mutations of TP53 and/or deletions in chromosome 17p [del(17p)] are associated with markedly decreased survival and predict impaired response to chemoimmunotherapy. Targeted agents including ibrutinib, idelalisib, venetoclax, or obinutuzumab may be of interest, if clinically indicated (24497559; NCCN Guidelines, Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, Version 2.2023).

Please note, according to WHO-HAEM-5 TP53 mutations can be considered multihit if either 2 different TP53 mutations (above 2% VAF), a single TP53 mutations with VAF >60%.

A mutation in NOTCH1 (p.(Pro2514ArgfsTer4)) was detected in this patient's sample.

NOTCH1 mutations have been reported to be diagnostically recurrent in 4-15% of CLL/SLL, and incidence of this mutation has been noted to be higher (15-25%) among patients with fludararabine-refractory disease. Some updated prognostic models have associated NOTCH1 mutated CLL with intermediate risk categorization and 10 year survival rates of <50%, and an association with reduced efficacy for ofatumumab has also been reported (31919090; NCCN Guidelines, Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, Version 2.2023). NOTCH1 mutations have also been noted to be associated more frequently with relapse disease and inferior clinical outcomes (WHO Haematopoietic and Lymphoid Tissues, 2023).

The present sample analysis is NEGATIVE for evidence of high level Tumor Mutation Burden or high level microsatellite instability.

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LEGEND:	Likely Response for defined therapy	Unlikely Response for defined therapy	Unknown therapeutic response	Associated with increased survival	Associated with decreased survival	Investigational agent available
	✓	✗	?	↑	↓	

The present sample analysis is negative for high level Tumor Mutation Burden. Presence of >10 mutations / megabase has been reported to be therapeutically predictive of favorable clinical outcomes and responsiveness to immune checkpoint inhibitors, according to some studies (29658845; 30395155). TMB is an evolving biomarker, and consensus standardization for this biomarker remains an ongoing imperative (30664300). To date, the median TMB for all tumor types tested by OnkoSight Advanced in general, is approximately 3.9 mutations / megabase, and 1.6 mutations / megabase for lymphoid neoplasm, in particular. According to some large scale cohorts published in the primary literature, a median TMB value of 1.7 mutations / megabase has been reported for chronic lymphocytic leukemia (28420421).

The present sample analysis is also negative for high level microsatellite instability.

Clinical and pathologic correlation is required to interpret these findings.

DETAILED GENETIC INTERPRETATION

Alteration	Interpretation
NOTCH1 **p.(Pro2514ArgfsTer4)** **c.7541_7542del** **38% allele frequency** **Exon 34** **NM_017617.4**	p.(Pro2514ArgfsTer4) represents a frameshift mutation in exon 34 of NOTCH1. This variation results in a shift of the reading frame and hence a pre-mature stop to the protein coding sequence. This variant occurs within the PEST domain and has been frequently reported in various types of Lymphoid neoplasm (COSMIC). PEST domain mutations may interfere with ubiquitination-mediated NOTCH1 downregulation and result in prolonged half-life of the intracellular NOTCH1 fragment, NICD1, and increased NICD1 transcriptional activity, leading to gain of protein function (19445024). The NOTCH1 gene is located on chromosome 9q34.3. The gene encodes a transmembrane ligand-activated transcription factor that is involved in a variety of developmental processes including regulation of cell fate decisions. Dual role for NOTCH1 gene has been suggested, with both oncogenic and loss of function mutations described that are cell- and tissue-type context dependent (2290788). Activating mutations in NOTCH1, reported in lung cancers and some leukemias, target the heterodimerization domain and the regulatory PEST domain important for proteasomal degradation, and result in stabilization of activated protein and gain of function (2000775; 24166518; 23295735). Inactivating mutations affecting N-terminal EGF-like ligand binding domain and resulting in loss of function have been identified in some solid tumors (21798897; 22907887). Both missense, frameshift and nonsense somatic mutations have been reported.
TP53 **p.(Arg181Gly)** **c.541C>G** **49% allele frequency** **Exon 5** **NM_000546.5**	p.(Arg181Gly) represents a missense mutation in exon 5 of TP53 at amino acid 181 converting the wild type residue, Arginine, into a Glycine p.(Arg181Gly) is located in the DNA-binding domain of TP53. This missense alteration is predicted to cause loss of function and is classified as a mutational hotspot residue (MSKCC). p.(Arg181Gly) is limited in a number of tumor samples (COSMIC) and has not been reported as a population variant in publicly available databases (gnomAD). The tumor protein p53 (TP53) is located on chromosome 17p13.1, and encodes a tumor suppressor protein. The TP53 protein mediates cellular response to DNA damage and is involved in a wide range of cellular processes, including transcriptional regulation, cell cycle control, apoptosis, and DNA repair. The TP53 gene is the most frequently mutated gene in human cancers, with somatic mutations associated with unfavorable prognosis in many tumor types (24132290; 21045690). Majority of the inactivating mutations are missense mutations that target the core DNA-binding domain (DBD), disrupting the DNA-binding capacity of p53 protein and leading to loss of function of the wild-type protein (24651012). Nonsense mutations, frameshift mutations, and deletions have also been reported in various tumor types. Some TP53 mutations are thought to result in dominant negative inhibition of wild-type p53 protein activity including inhibition of apoptosis, or in gain of function leading to increased proliferation, migration, and genomic instability (24916693; 24651012). Somatic mutations in TP53 are associated with leukemic transformation, complex karyotype, and adverse outcome in a number of hematological malignancies, including chronic myeloproliferative neoplasms (MPN) (21288114; 24478400), myelodysplastic syndrome (MDS) (21714648), and acute myeloid leukemia (AML) (22186996). Somatic TP53 mutations are independently associated with inferior prognosis in acute lymphoblastic leukemia (ALL) (25013160; 24829203; 25790293). TP53 mutations also have prognostic value

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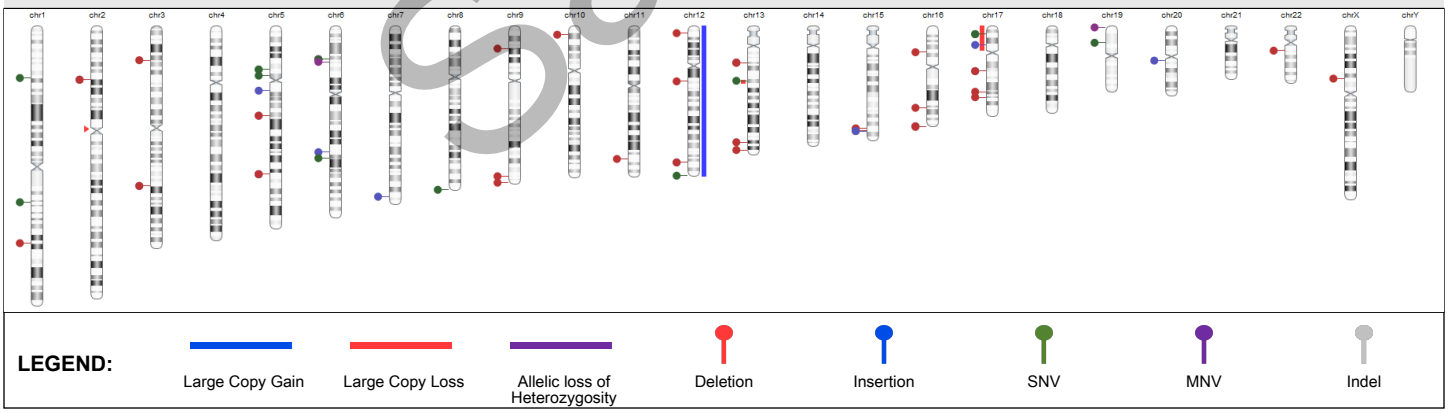
Alteration	Interpretation
	in common solid tumors (21045690). Pathogenic germline mutations in TP53 are associated with Li Fraumeni syndrome.
TP53 **p.(Val173Leu)** **c.517G>T** **9% allele frequency** **Exon 5** **NM_000546.5**	p.Val173Leu represents a missense mutation in exon 5 of TP53 converting the wild type amino acid Valine, into amino acid Leucine at residue 173. This mutation has been reported in a variety of tumor types (18410249; COSMIC), and has not been observed as a population variant in public genomic databases (EVS; ExAC). This substitution targets a residue located in the L2 loop of the DNA-binding domain (DBD) and has been shown to have a transforming activity in vitro (18410249; 10229196; 8001119). The tumor protein p53 (TP53) is located on chromosome 17p13.1, and encodes a tumor suppressor protein. The TP53 protein mediates cellular response to DNA damage and is involved in a wide range of cellular processes, including transcriptional regulation, cell cycle control, apoptosis, and DNA repair. The TP53 gene is the most frequently mutated gene in human cancers, with somatic mutations associated with unfavorable prognosis in many tumor types (24132290; 21045690). Majority of the inactivating mutations are missense mutations that target the core DNA-binding domain (DBD), disrupting the DNA-binding capacity of p53 protein and leading to loss of function of the wild-type protein (24651012). Nonsense mutations, frameshift mutations, and deletions have also been reported in various tumor types. Some TP53 mutations are thought to result in dominant negative inhibition of wild-type p53 protein activity including inhibition of apoptosis, or in gain of function leading to increased proliferation, migration, and genomic instability (24916693; 24651012). Somatic mutations in TP53 are associated with leukemic transformation, complex karyotype, and adverse outcome in a number of hematological malignancies, including chronic myeloproliferative neoplasms (MPN) (21288114; 24478400), myelodysplastic syndrome (MDS) (21714648), and acute myeloid leukemia (AML) (22186996). Somatic TP53 mutations are independently associated with inferior prognosis in acute lymphoblastic leukemia (ALL) (25013160; 24829203; 25790293). TP53 mutations also have prognostic value in common solid tumors (21045690). Pathogenic germline mutations in TP53 are associated with Li Fraumeni syndrome.

GENOME-WIDE DISTRIBUTION OF CNV AND SNV



ISCN Nomenclature:

seq[GRCh37] (12)x2~3,13q14.2q14.3(48037440_51922439)x1~2,17p13.3p11.1(1_22255105)x1~2

Patient Name: SAMPLE, PATIENT DOB: **Specimen ID: XXXXXXXXX**

Event	Cytoband	AMP Tier	Estimated Copy Number	Chromosome Region	Length (bp)	OMIM Genes Count
CN Gain	12p13.33 - q24.33	Tier I	2.65	chr12:1-133,851,895	133,851,895	882
CN Loss	13q14.2 - q14.3	Tier I	1.23	chr13:48,037,440-51,922,439	3,885,000	28
CN Loss	17p13.3 - p11.1	Tier I	1.27	chr17:1-22,255,105	22,255,105	285

CNV Interpretation Summary:

Trisomy 12 was detected.

Trisomy 12 is seen in approximately 20% of cases of CLL/SLL, and is associated with predominantly unmutated immunoglobulin variable region gene status. These cases demonstrate atypical morphological and immunophenotypic features, high proliferative rates. Patients with +12 CLL have an intermediate prognosis, and might show higher incidences of thrombocytopenia, Richter transformation, and other secondary cancers (29976738, (NCCN Guidelines, Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, Version 2.2023)).

Chromosome 13q loss involving RB1 region was detected.

Partial monosomy of chromosome 13q has been linked to the development of acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM).

In CLL/SLL, del(13q) as a sole abnormality is associated with favorable prognosis and the longest median survival (NCCN Guidelines, Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, Version 3.2023).

Deletion of TP53 (17p13) locus was detected.

Del(17p), with mutations in the remaining TP53 allele, is associated with short treatment-free interval, short median survival (32 months), and poor response to chemotherapy (11136261). Del(17p) is more frequently observed in patients with previously treated CLL, indicating acquisition and/or expansion of CLL clones with del(17p) during the course of treatment (NCCN Guidelines, Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, Version 3.2023).

Please note: *Sequencing-based copy number estimate should be interpreted in the context of tumor cellularity and ploidy. Enumerating copy numbers using a cell-based method such as in situ hybridization and/or Karyotype before any therapeutic decision is recommended.*

CLINICAL TRIALS

Context	NCTID	Title	Conditions	Location	Sponsor
General Consideration	NCT03204188	Ibrutinib, Fludarabine, and Pembrolizumab in High-Risk or Relapsed/Refractory Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma	Multiple Disease Types	Bethesda, Maryland, United States	National Heart, Lung, and Blood Institute (NHLBI)

MUTATIONAL HOTSPOTS

The following recurrently mutated codons demonstrated adequate sequencing coverage depths and show wild type sequences only.

Gene	Codons	Gene	Codons	Gene	Codons
BRAF	581, 594, 597, 600, 601	DNMT3A	882	EZH2	646
IDH1	132	IDH2	140, 172	KRAS	12, 13, 59, 60, 61
MYD88	265	NRAS	12, 13, 61	PIK3CA	81, 88, 93, 104, 106, 108, 111, 118, 542, 545, 546, 1043, 1044, 1047, 1049
SF3B1	623, 666, 700, 741	TP53	175, 213, 245, 248, 273, 282		

The following recurrently mutated codons demonstrated inadequate sequencing coverage depths (<100x), and the possibility of undersensitive detection cannot be excluded.

N/A

TRANSCRIPT ACCESSIONS FOR INTERROGATED GENES

Gene	Transcript ID	Gene	Transcript ID	Gene	Transcript ID
ATM	NM_000051.3	BCL2	NM_000633.2	BCL6	NM_001706.4
BIRC3	NM_182962.2	BRAF	NM_004333.4	BTK	NM_000061.2
CARD11	NM_032415.4	CD79B	NM_001039933.1	CDKN2A	NM_000077.4
CREBBP	NM_004380.3	CXCR4	NM_003467.2	DNMT3A	NM_022552.4
EP300	NM_001429.4	EZH2	NM_004456.4	IDH1	NM_005896.3
IDH2	NM_002168.3	IKZF1	NM_006060.5	IRF4	NM_002460.3
JAK1	NM_002227.2	JAK3	NM_000215.3	KMT2D	NM_003482.3
KRAS	NM_033360.3	MAP2K1	NM_002755.3	MEF2B	NM_001145785.2
MYC	NM_002467.4	MYD88	NM_002468.4	NOTCH1	NM_017617.4
NOTCH2	NM_024408.3	NRAS	NM_002524.4	PIK3CA	NM_006218.2
PIK3CG	NM_001282426.1	PLCG2	NM_002661.4	PTPRD	NM_002839.4
RHOA	NM_001664.3	SETD2	NM_014159.6	SF3B1	NM_012433.2
STAT3	NM_139276.2	STAT5B	NM_012448.3	TET2	NM_001127208.2
TNFRSF14	NM_003820.3	TP53	NM_000546.5		

COVERAGE DEPTH QC

Among the following targeted exons, >50% of coding sequences failed to achieve >100x coverage depths. Analytic sensitivity for potentially relevant genomic alterations may therefore be limited among the following interrogated loci:

Gene	Exons	Gene	Exons
NOTCH1	1	STAT5B	7

METHODS

Tissue microdissection and DNA isolation from tumor enriched areas are based on histologic review by an appropriately board certified pathologist; specimens with minimal tumor cellularity may be rejected. DNA is extracted and fragmented by Covaris shearing. DNA molecules from each sample are uniquely identified by ligation of a short oligonucleotide, sample specific barcodes. Each genomic DNA fragment is also tagged with a unique molecular identifier sequence (UMI) to collapse PCR duplicates and facilitate error corrected sequencing. Exons of 523 genes are enriched by hybridization to oligonucleotide synthetic probes, and PCR is performed to further amplify captured sequences. Amplified DNA is sequenced using Illumina sequencing-by-synthesis methodology. The assay interrogates whole exons and selected intronic regions across 523 genes to detect single base substitutions, insertion/deletions, and gene amplifications, targeting 1.94 million bases, encompassing 1.28 Mb of exonic sequence. The software requires a minimum number of 100 unique reads (after removal of PCR duplicates) to detect a mutation. An automated process that takes into account statistical confidence of base calling, alignment, and mapping quality, identifies variants (TSO500 Local App Software Release Notes V2.1.0; April 17, 2020). Following mapping of the read data to the human genome (reference build GRCh37/hg19), single nucleotide variants (SNVs), and insertion deletion events (Indels) with an allele frequency greater than 4% are detected. Detection of Insertions and Deletions larger than 29 bases have not been validated. 1.5x, 3x, and 5x fold changes have been validated with this assay to correspondent to high level FISH amplification for ERBB2, MET, and EGFR, respectively; fold changes for other genes are reported if in excess of 2.5x. Reported variants include known disease associated mutations and unclear variants with little or no literature support. Benign population polymorphisms or likely benign variants are not included in the report. Variant Tier categorizations are clinically reported in accordance with the AMP/ASCO/CAP consensus recommendations indicated in Li et. al. (27993330). Tumor Mutation Burden (TMB) is calculated as the number of mutations / megabase, and 1.94 megabases of genomic coding sequence are targeted for analysis. A cutoff of 10 mutations / MB is employed to report TMB as either high or low. Standardization for this biomarker remains an ongoing imperative, and further generation of assay specific, laboratory specific percentile cutoffs for individual tumor types has not yet been established. Median tumor mutation burden specific for tumor type is referenced from large scale patient cohorts in published studies (28420421). The assay interrogates 130 microsatellite regions to determine microsatellite instability class (MSI-Positive or MSI-Negative). Data from a minimum of 40 regions is needed to calculate an MSI score. A sample is classified as MSI-POSITIVE if 30% or more of the microsatellite regions are unstable (24310308; 29665853). Reportable Range: For full listings of interrogated genes please refer to: https://www.genpathdiagnostics.com/oncology/ngspersonalized-medicine/. Copy number alteration (CNA) events including deletions, duplications in excess of 25kb and copy neutral - loss of heterozygosity (CN - LOH) in excess of 10Mb are interrogated and reported if warranted and clinically relevant.

OnkoSight Advanced was developed and its performance characteristics were determined by GenPath, a division of BioReference Health, LLC. This test has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA has determined that such a clearance or approval is not necessary. Pursuant to the requirements of CLIA 88, this laboratory has established and verified the test's accuracy and precision. However, a false positive or false negative result incurred during any phase of the testing cannot be completely excluded. This assay does not detect balanced chromosomal abnormalities such as reciprocal translocations, Robertsonian translocations, inversions and balanced insertions or gene fusions. This assay does not determine variant causality, or whether a variant is inherited or somatically acquired. These results may be used for clinical or research purposes and therefore should be carefully considered within the context of other clinical and laboratory data. In the absence of an appropriate clinical context, the clinical utility of OnkoSight™ testing is not clearly defined. The information contained in this report reflects the current interpretation of the findings as of the date of the report, based on the available scientific information. This information, which comes from numerous sources, is subject to change over time in response to future scientific and medical findings and correlations. BioReference Health, LLC. makes no representation or warranty of any kind regarding the accuracy of information provided or contained in these manuscripts, references or other sources of information. If any of the information provided by or contained in the referenced material is later deemed to be inaccurate, this may impact the accuracy of this report and interpretation of the findings. BioReference Health, LLC. is not obligated to notify you of any impact that additional or modified information, or future scientific or medical research may have on this report. The laboratory is not responsible for reanalysis of the data or updated classification of this report or past reports' findings as the knowledge evolves. A medical provider can request a reassessment of clinical significance of variants and/or re-review of the clinical interpretation of the findings. Additional charges may apply for the updated report. Please contact the laboratory for more information if update is requested. This assay has been approved by the NYSDOH based on initial validation; orthogonal testing for full validation is currently ongoing. Please contact the laboratory for more information if update is requested.

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