OHSU Knight Cancer Institute
KNIGHT DIAGNOSTIC LABORATORIES

2525 SW 3rd Ave, STE 350
Portland, OR 97201
Phone: 855-535-1522 I Fax: 855-535-1329
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Patient Name:	Beaker, Donald	Accession #:	**19KD-274P0000**
Medical Record #:	03000000		
Account #:	2020202020		
Date of Birth:	1/11/1961		
Sex:	Male	Reference Lab no.:	
Referral Source:	Thomas J Done, MD PORTLAND HOSPITAL	Physician(s):	

GeneTrails Hematologic Malignancies NGS Panel

See Interpretation.

INTERPRETATION

Specimen ID: 19KD-274P0000
Sample Type: Bone marrow aspirate
Collection Date: 10/01/2019
Indication for Testing: anemia and thrombocytopenia; borderline dysplasia; possible MDS (versus benign)

GENOMIC ALTERATIONS

In this bone marrow from a patient with chronic cytopenias with a possible diagnosis of MDS, 3 pathogenic mutations in genes known to be involved in myeloid malignancies are observed, each at a moderate clonal burden. The presence of these 3 MDS-associated mutations would be consistent with a diagnosis of a clonal myeloid neoplasm – but is not sufficient to make a diagnosis. Correlation with clinical, cytogenetic, flow cytometric, and/or morphologic data would be required to reach a definitive diagnosis.

Variant(s) of Potential Clinical Significance (Tier II)

Gene: SRSF2
Variant: p.P95H (pathogenic hotspot mutation)
Variant allele frequency (VAF): 40%
Variant ID: COSM211029, COSM211504, COSM211505
SRSF2 (CCDS11749.1):c.284C>A; chr17:74732959G>T

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Codon P95 is a known mutation hotspot in SRSF2 in myeloid malignancies. The SRSF2 gene encodes a protein member of the spliceosome which is involved in assuring correct splicing of mRNA. SRSF2 mutations are common in MDS (~14%). Targeted therapy has not been reported for SRSF2 mutations.
SRSF2 mutations may portend a worse prognosis in MDS patients, especially with low risk MDS, although this may reflect an association of SRSF2 mutations with older age (Wu et al., Blood. 2012 Oct 11;120(15):3106-11Thol et al.,Blood. 2012 Apr 12;119(15):3578-84).

Gene: TET2
Variant: p.V1157fs*4 (loss of function)
Variant allele frequency (VAF): 39%
TET2 (CCDS47120.1):c.3468_3469del; chr4:106162553ATG>A

TET2 encodes a methylcytosine dioxygenase protein that plays a role in DNA methylation and regulation of transcription. TET2 is involved in myelopoiesis, and loss of function mutations in this gene are commonly found in MDS (20-25%). Targeted therapy has not been reported for TET2 mutations. However, TET2 mutations may predict a better response to hypomethylating agents in MDS.

Gene: RUNX1
Variant: p.R162K
Variant allele frequency (VAF): 31%
RUNX1 (CCDS13639.1):c.485G>A; chr21:36252877G>A

This RUNX1 mutation has been described many times in myeloid malignancies and is likely pathogenic. RUNX1 encodes the alpha subunit of the core binding factor (CBF transcription factor) and is involved in the regulation of normal hematopoiesis. RUNX1 mutations are very common in MDS (~10-20%), where they have been shown to confer a worse prognosis. Targeted therapy has not been reported for RUNX1 mutations.

Case reviewed by:
Richard Press, MD, PhD/Molecular Genetic Pathologist

Test Details:
This test is designed to detect alterations in a panel of 220 genes, many of which are known to play a role in leukemia and lymphoma pathogenesis, diagnosis, prognosis, response to therapy, disease

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monitoring, or inherited predisposition. All or selected coding exons and the canonical splice sites of the following genes are sequenced. No detectable alterations are identified in the following genes, unless otherwise listed above.

ABL1	AKT1	ANKRD26	ARID1A	ARID1B	ASXL1	ASXL2	ATG2B	ATM	ATRX
BCL2	BCL6	BCOR	BCORL1	BIRC3	BIRC6	BLM	BRAF	BRCA1	BRCA2
BRCC3	BRD4	BTK	CALR	CARD11	CASP10	CBL	CBLB	CBLC	CCND1
CCND3	CCR4	CD27	CD79A	CD79B	CDH11	CDKN2A	CDKN2C	CEBPA	CHD2
CHEK2	CREBBP	CRLF2	CSF1R	CSF3R	CTCF	CTLA4	CUX1	CXCR4	DAXX
DDX41	DDX54	DHX15	DHX29	DIS3	DNAH5	DNAH9	DNAJC21	DNM2	DNMT1
DNMT3A	DOCK8	DTX1	EED	EFTUD1	EGFR	ELANE	EP300	ERBB4	ETNK1
ETV6	EZH2	FAM47A	FAM5C	FAS	FAT1	FAT4	FBXO11	FBXW7	FLT3
FOXO1	FYN	GATA1	GATA2	GATA3	GNA13	GNAS	GNB1	GSKIP	HAX1
HIST1H1E	HNRNPK	HRAS	HVCN1	ID3	IDH1	IDH2	IGLL5	IKZF1	IKZF3
IL7R	IRF4	JAK1	JAK2	JAK3	KDM6A	KIT	KLF2	KLHL6	KMT2A
KMT2C	KMT2D	KRAS	LLGL2	LRRC4	LUC7L2	MAGT1	MAML1	MAP2K1	MECOM
MED12	MEF2B	MGA	MLH1	MPL	MSH2	MSH6	MYC	MYD88	NAF1
NBN	NF1	NFKBIE	NOTCH1	NOTCH2	NPAT	NPM1	NRAS	NT5C2	NXF1
PAX5	PCLO	PDGFRA	PHF6	PIGA	PIK3CD	PIM1	PLCG1	PLCG2	PMS2
POT1	PPM1D	PRDM1	PRKCB	PRPF40B	PRPF8	PRPS1	PSMB5	PTCH1	PTEN
PTPN11	RAD21	RB1	RBBP6	RELN	RHOA	RIT1	RPS15	RTEL1	RUNX1
RYR1	RYR2	SAMD9	SAMD9L	SAMHD1	SBDS	SETBP1	SETD2	SETDB1	SF1
SF3A1	SF3B1	SH2B3	SMARCA2	SMARCB1	SMC1A	SMC3	SOCS1	SPEN	SPI1
SRP72	SRSF2	STAG2	STAT3	STAT5B	STXBP2	SUZ12	SYK	SYNE1	TBL1XR1
TCF3	TCF4	TERC	TERT	TET2	TNFAIP3	TNFRSF14	TP53	TRAF3	U2AF1
U2AF2	UBR5	USH2A	VAV1	WAS	WHSC1	WT1	XPO1	ZBTB7A	ZRSR2

Low Limit of Detection:
The low limit of detection for this assay is 2% VAF at a minimal 900 read depth (5% at 700 read depth and 7% at 500 read depth). This case has an average read depth of 3467. However, a small fraction (100 minus the percentage in the parenthesis) of the targeted regions of the following genes: ASXL1 (99%), PCLO (99%), MED12 (99%), SPEN (99%), NOTCH2 (99%), JAK1 (99%), WAS (99%), ARID1B (98%), SETBP1 (98%), MAML1 (98%), STAT5B (94%), SPI1 (94%), KLF2 (93%), DTX1 (93%), BRD4 (92%), PMS2 (90%) have a higher low limit of detection of 10-15% VAF (if less than 250 read depth), or could harbor mutations that were missed by this analysis if the read depth is below 100. Further information on these low-coverage regions is available upon request. Low read counts may reflect changes in gene copy number or technical/stability issues with the sample.

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TIER CLASSIFICATION

Genomic variants have been classified in accordance with the 2017 standards and guidelines recommended by AMP/ASCO/CAP (1) and currently available resources. In brief, Tier I variants have strong evidence linking the genomic alteration to specific therapies, or are included in clinical practice guidelines or well-powered studies confirming their diagnostic, predictive, prognostic, and/or disease monitoring significance. Tier II variants have either targeted therapies available for different tumor types, ongoing preclinical or clinical trials of novel therapies, or have less strong evidence for their diagnostic, predictive, prognostic, and/or disease monitoring roles. Tier III variants (of unknown clinical significance) are neither observed in healthy populations nor have convincing published evidence of cancer association but cannot be classified as definitively benign or likely benign. We do not report benign or likely benign (Tier IV) variants. The classification of a variant is dependent on the specific clinical scenario, which could change in a different patient and/or in a new sample from the same patient, or if there are new data/information relevant to this variant.

METHOD(S)

Genomic DNA is extracted and purified from blood, bone marrow or other hematopoietic tissue from fresh or fixed samples. If the submitted sample is from FFPE, the specimen is examined microscopically and, if deemed helpful to enhance sensitivity, genomic DNA is extracted and purified from micro dissected, tumor-rich areas. Mutations are screened by massively parallel sequencing (next-generation sequencing) using a combination of multiplexed PCR (customized QIAseq targeted DNA panel with molecular barcodes) and sequencing on an Illumina platform (NextSeq 500 or 550). Sequencing data is then aligned against a reference genome [hg19]. An in-house bioinformatics analysis pipeline has been used that employs multiple established variant calling tools (FreeBayes, MuTect2, and Scalpel) and variant annotation tools (Oncotator). The assay is validated according to AMP guidelines (2,3).

With regard to insertions and deletions, this test is known to be biased toward detecting shorter alterations, and often to underestimate variant allele frequency (VAF) of these types of variants. Therefore, a supplementary non-biased size-based assay is concomitantly run to ensure that internal tandem duplication insertions in FLT3 exon 14 are not missed. Furthermore, a supplementary assay is concomitantly run to detect partial tandem duplications in KMT2A/MLL (aka MLL-PTD) if warranted by the clinicopathological findings of the patient. Sequencing often does not detect large deletions or duplications in other genes targeted on this panel. In addition, this test does not detect mutations in the regulatory regions, deep introns, or highly homologous regions containing pseudogenes and/or highly repetitive regions.

This assay is intended to detect somatically-acquired variants in cancer-associated genes and is not intended to detect germline variants for the diagnosis of inherited cancer predisposition syndromes. If an inherited variant is suspected, other sequencing tests may be indicated, and genetic counseling may be warranted.

REFERENCES

1. Li MM, et al; 2017, J Mol Diagn. 19(1):4-23.

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2. Jennings LJ, et al; 2017, J Mol Diagn. 19(3):341-365.
3. Roy S, et al; 2018, J Mol Diagn. 20(1):4-27.
4. NCCN guidelines: https://www.nccn.org/professionals/physician_gls/default.aspx
5. The 2017 ELN recommendations: Blood. 2017; 129(4):424-447.
6. The 2016 revision to the WHO classification: Blood. 2016; 127(20):2375-405.
7. Catalogue Of Somatic Mutations In Cancer: http://cancer.sanger.ac.uk/cosmic
8. ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
9. JAX-Clinical Knowledgebase (CKB): https://ckb.jax.org/
10. CIViC: https://civicdb.org/home

DISCLAIMER

This test was developed and its performance characteristics determined by the OHSU Knight Diagnostic Laboratories. It has not been cleared or approved by the Food and Drug Administration. FDA approval is not required for the clinical use of the test, and therefore validation was done as required under the requirements of the Clinical Laboratory Improvement Act of 1988 (CLIA). The OHSU Knight Diagnostics Laboratories are fully licensed by the state of Oregon under CLIA and are accredited by the College of American Pathologists (CAP). Laboratory Director: Christopher Corless, M.D., Ph.D.

Reviewed and electronically signed by RICHARD D PRESS, MD,PhD
10/1/2019 11:26 AM

Bone marrow specimen 19KD-274P0000 from Bone marrow Unspecified. Ordered by Ed P Opal, MD. Authorized by Ed P Opal, MD. Collected: 10/1/2019 1102 Received: 1102. Verified: 10/1/2019 1126. Resulted by OHSU-KNIGHT DIAGNOSTIC LABORATORIES.

Specimen Details

Submitter ID	Tests
19KD-274P0000	GeneTrails Hematologic Malignancies NGS Panel

Submitter

PORTLAND HOSPITAL	4800 NE HOSPITAL WAY, PORTLAND Oregon 97213

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