Patient ID	Patient Name		Birth Date	Sex	Age
SA00164234	TESTING, NGSHM ABNORMAL		2001-01-01	F	22
Order Number	Client Order Number	Ordering Physician	Report Notes		
SA00164234	SA00164234	CLIENT,CLIENT			
Account Information		Collected			
C7028846 DLMP Rochester		25 Oct 2023 00:00			

Myeloid Neoplasms, NGS, V

Specimen Type

MCR

Bone marrow

Indication for Test

MCR

AML

Pathogenic Mutations Detected

MCR

1. FLT3: Chr13(GRCh37):g.28608217_28608309dup;
NM_004119.2(FLT3):c.1747_1837+2dup; p.? (FLT3-ITD, 40%, estimated ITD to wild-type allelic ratio 0.67 by this NGS assay, see interpretation*)

Note: Alterations in genes with FDA-Approved Therapies for Acute Myeloid Leukemia FLT3: Detected IDH1: Not Detected IDH2: Not Detected

No other pathogenic mutations were detected in the other genes tested on the panel at the reportable limit of assay detection. See below for Variants of Unknown Significance and Additional Notes. Please see the section of "Panel Gene List" below for the complete list of genes tested.

Interpretation

MCR

1. FLT3: Chr13(GRCh37):g.28608217_28608309dup;
NM_004119.2(FLT3):c.1747_1837+2dup; p.?

Normal gene/protein function: The FLT3 gene located on chromosome 13q12 encodes the protein FMS-like receptor tyrosine kinase 3 (FLT3), a class III receptor tyrosine kinase (RTK) that plays an important role in hematopoietic stem cell survival, proliferation and differentiation (Kiyoi and Naoe, 2002, 12400596). FLT3 activates downstream signaling pathways including RAS, AKT1, ERK and mTOR (Mizuki et al., 2000, 11090077; Zhang et al., 1999, 10080542; Chen et al., 2010, 21067588).

Mutation Effect: The c.1747_1837+2dup is a FLT3-internal tandem duplication (FLT3-ITD) mutation. FLT3-ITDs constitutively activate the receptor tyrosine kinase function, leading to abnormal proliferative cell signaling (Kiyoi et al, 2002, 12400596).

Disease associations: FLT3-ITD occurs frequently (20–30%) in acute myeloid leukemia (AML). FLT3-ITDs are associated with adverse prognosis in AML, particularly in patients with normal or intermediate risk karyotype (Frohling et al., 2002, 12393388; Schlenk et al., 2008, 18450602; Patel et al., 2012, 22417203; Estey et al., 2013, 23526416; Levis et al., 2013, 24319184). FLT3-ITD occurs rarely in myelodysplastic syndrome (MDS, <1%) and chronic myelomonocytic leukemia (CMML, approximately 3%) and does not adversely impact clinical outcome (Daver et al., 2013, 23115106). *A FLT3-ITD allelic ratio cut-off value of 0.5 as determined by a fragment analysis (such as FLT3 Mutation Analysis, test ID: FLT) was recommended by the European LeukemiaNet to classify FLT3-ITD in AML risk stratification (Dohner, et al, 2017, 27895058). Clinical correlation is recommended.

Therapeutic implications: For adult acute myeloid leukemia patients harboring a FLT3-ITD and/or TKD (tyrosine kinase domain) mutation,

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C7028846 DLMP Rochester		25 Oct 2023 00:00			

midostaurin was approved by the FDA for in combination therapy with standard cytarabine and daunorubicin induction, and cytarabine consolidation in newly diagnosed patients. Gilteritinib was also approved in refractory or relapsed AML patients. FDA has recently approved quizartinib with standard cytarabine and anthracycline induction and cytarabine consolidation, and as maintenance monotherapy following consolidation chemotherapy, for adult AML patients harboring FLT3-ITD mutation(s). Multiple FLT3 inhibitors are undergoing evaluation in clinical studies.

Variants of Unknown Significance

MCR

None

The VUS variants listed here (with approximate variant allele %) are not sufficiently characterized in the current literature and are therefore of uncertain clinical significance at this time. They are reported here for future reference in the event they become clinically significant in the light of new scientific data.

Clinical Trials

MCR

Information regarding possible clinical trials for this patient can be found at the following sites:

1). ClinicalTrials.gov:
http://clinicaltrials.gov/ct2/search/advanced
2). Mayo Clinic:
http://www.mayo.edu/research/clinical-trials
3). National Cancer Institute:
http://www.cancer.gov/clinicaltrials/search
4). The Leukemia and Lymphoma Society's Clinical Trial Support Center https://www.hematology.org/education/clinicians/clinical-trial-support-center

Additional Notes

MCR

None

Method Summary

1 MCR

DNA is extracted from peripheral blood or bone marrow specimens. Library preparation for Next Generation Sequencing (NGS) is performed followed by probe hybridization and capture. Sequencing of the final sample library is performed on a NGS instrument. Following bioinformatic processing of the sequencing data, the sequencing results are interpreted to provide a final clinical report. Genomic alterations are called according to human genome reference build GRCh37 (hg19).

Performance characteristics of NGS panel:
Single base substitutions: accuracy >99%; reproducibility 100% (intra- and interassay); sensitivity 2–4% variant allele fraction with a minimum depth of coverage of 500X. Small insertion/deletion events (up to 500 bp): accuracy >99%; reproducibility 100% (intra- and interassay); sensitivity 2–4.99% variant allele fraction with a minimum depth of coverage of 500X. Larger single gene insertion/deletion events with sizes between ≥ 501 bp and ≤ 5 kb with a variant allele fraction ≥ 5% and minimum depth of coverage of 500X* will be reported. Variants involving multiple genes or single gene events ≥ 1 kb with variant allele fractions between 2–5% will not be reported. Events ≥ 5 kb with a variant allele fraction ≥ 5% within a single gene may be identified and if present, general information on gene,

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chromosome, and event type will be provided in the Additional Notes section, with suggestion for confirmatory testing. *Some genetic or genomic alterations such as very large insertion/deletion events, copy number alterations (CNA) and gene translocation events are not detected by this assay.

Disclaimer

MCR

CLINICAL DISCLAIMER

The finding of a genetic alteration does not necessarily indicate the presence of a myeloid neoplasm. Some apparent mutations classified as VUS may represent very low population frequency polymorphisms. Hematopoietic cells in some individuals may have age-related genetic alterations associated with myeloid neoplasms in the absence of a hematologic malignancy (clonal hematopoiesis of indeterminate potential, CHIP, also known as age-related clonal hematopoiesis, ARCH). In addition, patients with unexplained cytopenias may also harbor similar myeloid neoplasm-associated gene mutations (clonal cytopenias of uncertain significance, CCUS) [PMIDs: 25931582, 30482770, 31672865, and 30759825]. Distinction between CHIP or CCUS and a myeloid malignancy requires correlation with clinical, pathologic, and other laboratory findings. Prior treatment for hematologic malignancy could affect the results obtained in this assay. In particular, prior allogeneic hematopoietic stem cell transplant (HSCT) may cause difficulties in resolving somatic or polymorphic alterations, or in assigning variant calls correctly to donor and recipient fractions, if pertinent clinical or laboratory information (e.g. chimerism engraftment status) is not provided. This assay does not distinguish between somatic and germline alterations in analyzed gene regions, particularly with VAF near 50% or 100%. If nucleotide alterations in genes associated with germline mutation syndromes are present and there is also a strong clinical suspicion or family history of malignant disease predisposition, additional genetic testing and appropriate counseling may be indicated. This report interpretation is based on current medical and scientific literature, but clinical significance may not be completely established for all reported target gene abnormalities identified. Correlation with clinical, histopathologic and additional laboratory findings is required for final interpretation of these results. The final interpretation of results for clinical management of the patient is the responsibility of the managing physician.

TECHNICAL DISCLAIMER

The depth of sequencing coverage may be variable for some target regions, but assay performance below the minimum acceptable criteria, or for failed regions are noted. Analysis of rare (low allele frequency) polymorphisms may be problematic in some cases. A low tumor cell percentage in the sample may affect the true mutation VAF and/or sensitivity. Suboptimal-performing regions (i.e. less than the expected minimum depth of coverage) may affect analytic sensitivity for detecting lower level mutations. This is a qualitative test. The variant read fractions are provided for information only and represent a relative proportion of mutated alleles, but do not indicate a measure of analytical sensitivity for the given genes; assay sensitivity is as stated in the method summary. Some genetic or genomic alterations, such as very large insertion/deletion events, copy number alterations (CNA) and gene translocation events are not detected by this assay.

OncoHeme Panel Gene list

MCR

ANKRD26 (NM_014915.2) 5'UTR, start at c.–172, exons 1–4, ASXL1 (NM_015338.5) exons 10–13, BCOR (NM_001123385.1) exons 4–15, BCORL1 (NM_001184772.2) exons 1–13, BRAF (NM_004333.4), exons 11 and 15, CALR (NM_004343.3) exon 9, CBL (NM_005188.3) intron 7 last 100bp before start of exon 8, exon 8, intron 8, and exon 9, CEBPA (NM_004364.4) exon 1, CSF3R (NM_000760.3) exons 4, 13–14 and 17, DDX41 (NM_016222.2) exons 1–17, DNMT3A (NM_022552.4) exons 8–23, ELANE (NM_001972.2) exons 1–5, ETNK1 (NM_018638.4) exons 2–5, ETV6 (NM_001987.4) exons 3–8, EZH2 (NM_004456.4) exons 2–20, FLT3 (NM_004119.2) exons 14–20 and intron 14, GATA1 (NM_002049.3) exons 2 and 4, start at c.–19–30 before exon 2, GATA2 (NM_032638.4) exons 1–6, intron 4, c.1017+1 - 1017+870, IDH1 (NM_005896.3) exons 4, 6–8, IDH2 (NM_002168.3) exons 3–4, 6–8, JAK2 (NM_004972.3) exons 12–20, KDM6A (UTX) (NM_021140.3) exons 1–29, KIT (NM_000222.2) exons 8–11 and 17, KRAS

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C7028846 DLMP Rochester		25 Oct 2023 00:00			

(NM_033360.3) exons 2–4, MPL (NM_005373.2) exons 1–12, NF1 (NM_001042492.2), exons 1–58, NPM1 exons 9–11, intron 10 start 3 bp before exon 11, NRAS (NM_002524.4) exons 2–4, PHF6 (NM_001015877.1) exons 2–10, PPM1D (NM_003620.3) exons 1–6, PTPN11 (NM_002834.3) exons 3–4 and 12–13, RAD21 (NM_006265.2) exons 1–2, 4–7, 9–11, 13–14, intron 9 start 3 bp before exon 10, RUNX1 (NM_001754.4) exons 1–9, intron 7 start 13 bp before exon 8, SETBP1 (NM_015559.2) partial exon 4; amino acids 400–950, SH2B3 (LNK) (NM_005475.2) exon 2–8, SF3B1 (NM_012433.2) exons 13–16, SMC3 (NM_005445.3) exons 7–8, 13, 17, 19, 21, and 29, SRSF2 (NM_003016.4) exons 1–2, STAG2 (NM_001042750.1) exons 4–34, exons 12 and 17 start at -3 in preceding introns, STAT3 (NM_139276.2), exons 2–24, TERT (NM_198253.2) exons 2–16, TET2 (NM_001127208.2) exons 3–11, TP53 (NM_000546.4) exons 4–11, U2AF1 (NM_001025203.1) exons 2, 6, and 8, UBA1 (NM_003334.3) exons 2–26, WT1 (NM_024426.2) exons 1–10, and ZRSR2 (NM_005089.3) exons 1–11.

Unless otherwise noted, intronic coverage surrounding exons is ±10 bp.

For some genes, the transcript IDs used in this analysis may differ from those present in cancer mutation databases or other similar sources.

Variants will be reported out using HGVS nomenclature per the documentation version available at varnomen.hgvs.org at the time of the report.

Reviewed By: MCR

Signing Pathologist: LAUREN SCHULTZ

NGSHM Result MCR

See Interpretation

Received: 25 Oct 2023 10:09 **Reported:** 25 Oct 2023 10:19

Laboratory Notes

1 This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

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