Patient ID	Patient Name		Birth Date	Sex	Age
SA00154846	TESTINGRNV, VALIDATION M		1999-09-09	M	23
Order Number	**Client Order Number**	**Ordering Physician**	**Report Notes**		
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Account Information		**Collected**			
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Plasma Cell Myeloma, NGS, V

Specimen Type

MCR

Bone marrow

Indication for Test

MCR

Multiple myeloma

Pathogenic Mutations Detected

MCR

1. SF3B1: Chr2(GRCh37):g.198266834T>C;
NM_012433.2(SF3B1):c.2098A>G; p.Lys700Glu (35%)

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

Mutation effect: The missense c.2098A>G (p.K700E) variant occurs commonly in hematologic neoplasms and accounts for more than half of somatic SF3B1 mutations. Mutations in SF3B1 contribute to tumorigenesis in hematologic malignancies through induction of abnormal transcription and alternative gene splicing (Boultwood et al., 2014, 24080589; Gazzola et al., 2013, 23160465; Yoshida et al., 2011, 21909114).

Disease associations: Somatic mutations in SF3B1 have been identified in both myeloid and lymphoid tumors with similar mutation distribution (Gazzola et al., 2013, 23160465; Wan and Wu, 2013, 23568491; Papaemmanuil et al., 2011, 21995386; Malcovati et al., 2011, 21998214). In chronic lymphocytic leukemia/small lymphocytic lymphoma (GLUSLL), SF3B1 mutations are seen in approximately 4–12% of cases and are more frequent (17–24%) with disease progression. They are associated with shorter time to treatment, fludarabine-refractory disease, and inferior overall survival (Wan et al., 2013, 23568491; Gazzola et al., 2013, 23160465; Jeromin et al., 2014, 241134 72; Rossi et al., 2011, 22039264; Nadeu et al., 2015, 26837699; Wang et al., 2011, 22150006). Additionally, SF3B1 is mutated in 20–28% of myelodysplastic syndrome (MOS) and is particularly prevalent (>80%) in MOS with ring sideroblasts (MOS-RS) and the myeloproliferative/ myelodysplastic neoplasm refractory anemia with ring sideroblasts associated with marked thrombocytosis (MDS/MPN-RS-T)(Brosius et al.,2013, 23594705; Patnaik and Tefferi, 2015, 25899435; Malcovati et al., 2015, 25957392). The majority of studies showed a good prognosis in SF3B1 -mutated low-risk MOS (Malcovati et al., 2015, 25957392; Malcovati et al., 2014, 24970933; Pellagatti et al., 2015, 25645650; Gazzola et al., 2013, 23160465; Patnaik and Tefferi, 2015, 25899435). SF3B1-mutant MOS has been proposed as a distinct disease subtype by the International Working Group for the Prognosis of MOS with specified diagnostic and exclusion criteria (Malcovati et al., 2020, 32347921).

Therapeutic implications: The food and Drug Administration (FDA) has improved Bruton's tyrosine kinase (BTK) inhibitors (e.g., ibrutinib and acalabrutinib), PI3K inhibitors (e.g., idelalisib and duvelisib) and BLC-2 inhibitor venetoclax for CLUSLL and/or some other low-grade B-cell lymphoma patients. Currently, there is no approved splicing modulators for hematologic neoplasms. In pre-clinical studies, splicing modulator H3B-88OO preferentially killed SF3B1-mutant cells and induced antitumor activity in SF3B1 -mutant xenograft leukemia models (Seiler et al., 2018, 29457796). Another splicing modulator E71O7 inhibited the growth of primary human CLL cells, increased BCL-2

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dependency, and sensitized primary human CLL cells and murine venetoclax-resistant CLL-like cells to venetoclax treatment (Agrawal et al., 2017, 292967 42; Hacken et al., 2018, 30282833). Phase 1 clinical trial of H3B-88OO in myeloid neoplasms showed dose-dependent target engagement and decreased RBC or platelet transfusion requirements in 1% of patients in a relatively small study cohort (n=84) although no objective complete remission or partial remission was achieved (https://doi.org/10.1182/blood-2019-123854). Additional information about available clinical trials can be found at ClinicalTrials.gov.

Variants of Unknown Significance

MCR

None.

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

Additional Information

MCR

None.

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

Method Summary:

1 MCR

DNA is extracted from validated specimen sources. Fresh bone marrow aspirate specimens are enriched for plasma cells by a flow cytometric cell sorting method prior to DNA extraction. 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 >99% (intra- and interassay); sensitivity 5% variant allele fraction with a minimum depth of coverage of 500X.

Insertion/deletion events: accuracy 100%; reproducibility >99% (intra- and interassay); sensitivity 5% variant allele fraction with a minimum

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depth of coverage of 500X. 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

Mutation calls detected between 5–10% variant allele fractions (VAF) may indicate low-level (i.e. subclonal) tumor populations, although the clinical significance of these findings may not be clear. Some apparent mutations classified as VUS may represent rare or low frequency polymorphisms. 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. Multiple additional factors may also impact the observed VAF including copy number alterations of the genetic locus, chromosome ploidy changes or subclonal composition. Correlation with clinical, histopathologic and additional laboratory findings is required for final interpretation of these results. 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. The final interpretation of results for clinical management of the patient is the responsibility of the managing physician. At this time, it is not standard practice for the laboratory to systematically review likely pathogenic variants or variants of uncertain significance that have been previously detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time.

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 is 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. In the rare event of suboptimal measured DNA input quantity for library preparation (e.g. due to limited tissue amount), detection sensitivity for subclonal variants near the assay limit of detection may be decreased, despite the presence of otherwise acceptable sequencing quality metrics. 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.

Panel Gene List

MCR

BIRC3 (NM_001165.4) Exons 2–9, BRAF (NM_004333.4) Exons 11–18, CCND1 (NM_053056.2) Exons 1–5, CDKN2A (NM_000077.4) Exons 1–3, CRBN (NM_016302.3) Exons 1–11, CUL4A (NM_001008895.2) Exons 2–20, CUL4B (NM_003588.3) Exons 2–22, CXCR4 (NM_003467 .2) Exons 1–2, DIS3 (NM_014953.4) Exons 1–21, EGFR (NM_005228.3) Exons 1–28, TENT5C (NM_017709.3) Exon 2, IDH1 (NM_005896.3) Exon 4, IDH2 (NM_002168.3) Exon 4, IKZF1 (NM_006060.6) Exons 2–8, IKZF3 (NM_012481.4) Exons 1–8, KRAS (NM_033360.3) Exons 2–4, MYC (NM_002467.4) Exons 1–3, MYD88 (NM_002468.4) Exons 1–5, NRAS (NM_002524.4) Exons 2–4, NSD2 (NM_001042424.2) Exons 17–20, PIK3CA (NM_006218.2) Exons 2–11, 14–21, PIM1 (NM_001243186.1) Exons 1–6, STAT3 (NM_ 139276.2) Exons 2–24, TP53 (NM_000546.4) Exons 4–11, TRAF3 (NM_ 145725.2) Exons 3–12, XBP1 (NM_001079539.1) Exons 1–6.

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For some genes, the transcript IDs used in the analysis may not be the same as in other cancer mutation databases, such as COSMIC http:/ /cancer.sanger.ac.uk/cosmic

Reviewed By: MCR

Signing Pathologist: BENJAMIN BAILEY

NGPCM Result MCR

See Interpretation

Received: 14 Oct 2022 11:34 **Reported:** 14 Oct 2022 12:25

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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