Client: ARUP Example Report Only
500 Chipeta Way
Salt Lake City, UT 84108
UNITED STATES

Physician: EXAMPLE, PHYSICIAN

Patient: MYE CNV, pos example

DOB	
Sex:	Unknown
Patient Identifiers:	54049
Visit Number (FIN):	54437
Collection Date:	10/26/2023 12:21

Myeloid Malignancies Mutation and Copy Number Variation Panel by Next Generation Sequencing

ARUP test code 3016621

MYE CNV Proposed Diagnosis	AML unspec
MYE CNV Specimen	Whole Blood
MYE CNV Interp	See Note

Myeloid Mutation Panel by NGS, DelDup

Submitted diagnosis or diagnosis under consideration for variant interpretation: Acute myeloid leukemia, unspecified (AML, unspec)

Section 1: Molecular Variants

TIER 1: Variants of Known Clinical Significance in Hematologic Malignancies

1. FLT3 c.1799_1800ins60, p.Thr582_Leu601dup (NM_004119.3)
VAF: Not Reported
Two distinct FLT3 mutations are detected in trans configuration (on separate chromosomes). FLT3 encodes a receptor tyrosine kinase involved in regulating the development of hematopoietic stem cells (33). This variant is a FLT3 internal tandem duplication (FLT3-ITD). FLT3-ITD mutations occur in the juxtamembrane domain and are found in 20-30% of acute myeloid leukemia (AML) patients (7) (29) (35). AML patients with FLT3-ITD mutations have a worse outcome (shorter overall survival and higher relapse risk) compared to patients without FLT3-ITD mutations (7) (14) (30). The prognostic value of FLT3-ITD mutations in AML patients also depends on the mutation status of other prognostic markers (14) (29) (30) (31). One study showed that AML patients with mutated DNMT3A, mutated NPM1, and FLT3-ITD had a worse outcome compared to patients with any two of these three genes mutated (26). A meta-analysis showed that patients with FLT3-ITD and NPM1 mutations have improved complete remission, disease-free survival, and overall survival compared with those who only have FLT3-ITD, although this is inferior to NPM1 mutation alone (20). The variant allele frequency for a FLT3-ITD may not be representative of the FLT3-ITD allelic ratio and is not reported.

2. FLT3 c.1780_1781ins30, p.Asp593_Phe594ins10 (NM_004119.3)
VAF: Not Reported
This second FLT3 mutation is also an internal tandem duplication. The variant allele frequency for a FLT3-ITD may not be representative of the FLT3-ITD allelic ratio and is not reported.

H=High, L=Low, *=Abnormal, C=Critical

3. WT1 c.1114-3_1132del, p.? (NM_024426.6)
VAF: 82.3 %
Two distinct WT1 mutations are detected in trans configuration (on separate chromosomes). WT1 encodes Wilms Tumor 1 (WT1), a transcription factor that functions as both a tumor suppressor and oncogene (11) (39). Somatic mutations of WT1 are found in 6-8% of patients with AML (12) (17) (21) (32) and are rare in patients with MDS (32). In AML, WT1 mutations are often frameshift and nonsense variants (12). This particular splice-site mutation abolishes the splice acceptor site of intron 6 and is predicted to cause abnormal splicing of WT1 (Alamut Visual software v.2.11.0). The prognostic impact of WT1 mutations in AML patients is uncertain. One study found that WT1 mutations did not correlate with overall survival in cytogenetically normal AML patients (6), whereas other studies have found that AML patients with WT1 mutations have an inferior prognosis (12) (28) (37). In one study of a large cohort of AML patients, WT1 mutations did not correlate with prognosis in the overall cohort but did correlate with shorter event-free survival in cytogenetically normal AML patients (17). WT1 mutations have also been reported to co-occur with FLT3-ITD mutations (1) (18). Please note that the variant allele frequency is high due to copy neutral loss of heterozygosity (CN-LOH) of the WT1 locus on chromosome 11.

4. WT1 c.1147_1148dup, p.Val384fs (NM_024426.6)
VAF: 5.8%
This second WT1 mutation is predicted to alter the normal function of WT1.

5. SRSF2 c.284C>G, p.Pro95Arg (NM_003016.4)
VAF: 46.4%
SRSF2 encodes a component of the RNA splicing complex known as the spliceosome. Somatic mutations of SRSF2 are found in 1-6% of patients with de novo AML, in 7-24% of patients with secondary AML (22) (26) (40) (41), and in approximately 10% of therapy-related AML patients (19). In myeloid malignancies, acquired SRSF2 mutations commonly affect codon Pro95 (22). This particular mutation is recurrent in myeloid malignancies (3). SRSF2 mutations are associated with decreased overall survival and disease-free survival in patients with de novo AML (13) (26). SRSF2 mutations predict more frequent progression to secondary AML in patients with MDS (36) and correlate with shorter overall survival in these patients.

6. ASXL2 c.1840C>T, p.Arg614* (NM_018263.6)
VAF: 41.8%
ASXL2 encodes an epigenetic regulator of gene expression (15). Somatic ASXL2 mutations are found in 18-23% of AML patients with t(8;21)(q22;q22) (also known as core-binding factor AML) (4) (23). ASXL2 mutations are typically frameshift and nonsense alterations (4) (23). This particular mutation is predicted to alter the normal function of ASXL2. ASXL2 mutations do not predict overall survival but may be associated with an increased incidence of relapse in AML patients with t(8;21); however, the different relapse rates did not reach statistical significance in this study (23). Correlation with cytogenetic findings may be helpful, if available.

7. GATA2 c.1140C>G, p.His380Gln (NM_032638.5)
VAF: 7.3%
GATA2 belongs to the GATA family of transcription factors and regulates hematopoiesis through two conserved zinc finger domains. Overall, somatic GATA2 mutations are found in 1-4% of patients with sporadic myeloid malignancies (19) (26) (27). GATA2 mutations are common in adult AML patients with biallelic CEBPA mutations, but are rare in adult AML patients with wild-type CEBPA (5) (9) (10). Pathogenic germline variants of GATA2 cause a range of hematopoietic defects, including MonoMAC

syndrome, dendritic cell, monocyte, B and NK lymphoid deficiency syndrome (DCML), familial MDS, AML, and blast transformation in chronic myeloid leukemia (CML) (2) (34). Somatic GATA2 mutations in hematological malignancies are typically missense mutations within the N-terminal zinc-finger domain and in-frame deletions/insertions in the C-terminal zinc-finger domain (16) (24) (25). Somatic frameshift and nonsense mutations in GATA2 are generally detected outside of the zinc-finger domains (24). This particular mutation has been reported in hematological malignancies (8) (38). In AML patients, GATA2 mutations are confined to the N-terminal zinc finger domain, and frequently co-occurred with biallelic CEBPA, KIT and FLT3 mutations (24). Some studies found that GATA2 mutations had no impact on the clinical outcome in CEBPA-double/FLT3-ITD-negative AML patients (9). Another study found that GATA2 mutations were associated with favorable prognosis in intermediate-risk karyotype AML with biallelic CEBPA mutations (5). The prognostic significance of GATA2 mutation in the absence of CEBPA mutation is unclear.

TIER 2: Variants of Unknown Clinical Significance in Hematologic Malignancies

None found

Section 2: Copy Number Variants and CN-LOH

TIER 1: Variants of Known Clinical Significance in Hematologic Malignancies

1. CN-LOH 11p15.5p13
VAF: 85%

TIER 2: Variants of Unknown Clinical Significance in Hematologic Malignancies

1. CN-LOH 1q42.13q44
VAF: 76%

Copy Number Variants and CN-LOH Interpretation
The above tier 1 copy number variants (CNVs) and/or copy-neutral loss of heterozygosity (CN-LOH) are either recurrent findings in hematologic malignancies or clonal changes in neoplastic processes.

CNV/CN-LOH Variant Nomenclature:
seq[GRCh37] 11p15.5p13(193865_33856444)x2 mos hmz
seq[GRCh37] 1q42.13q44(228532195_248571228)x2 mos hmz

References
1: Becker H, Marcucci G, Maharry K et al, Mutations of the Wilms tumor 1 gene (WT1) in older patients with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. Blood 2010. PMID:20442368
2: Collin M, Dickinson R, Bigley V, Haematopoietic and immune defects associated with GATA2 mutation. Br J Haematol 2015. PMID:25707267
3: COSMIC: https://cancer.sanger.ac.uk/cosmic
4: Faber ZJ, Chen X, Gedman AL et al, The genomic landscape of core-binding factor acute myeloid leukemias. Nat Genet 2016. PMID:27798625
5: Fasan A, Eder C, Haferlach C et al, GATA2 mutations are frequent in intermediate-risk karyotype AML with biallelic CEBPA mutations and are associated with favorable prognosis. Leukemia 2013. PMID:22814295
6: Gaidzik VI, Schlenk RF, Moschny S et al, Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. Blood 2009. PMID:19221039
7: Gilliland DG, Griffin JD, The roles of FLT3 in hematopoiesis and leukemia. Blood 2002. PMID:12176867

8: Gionfriddo I, Brunetti L, Mezzasoma F et al, Dactinomycin induces complete remission associated with nucleolar stress response in relapsed/refractory NPM1-mutated AML. Leukemia 2021. PMID:33654209
9: Green CL, Tawana K, Hills RK et al, GATA2 mutations in sporadic and familial acute myeloid leukaemia patients with CEBPA mutations. Br J Haematol 2013. PMID:23560626
10: Greif PA, Dufour A, Konstandin NP et al, GATA2 zinc finger 1 mutations associated with biallelic CEBPA mutations define a unique genetic entity of acute myeloid leukemia. Blood 2012. PMID:22649106
11: Hohenstein P, Hastie ND, The many facets of the Wilms' tumour gene, WT1. Hum Mol Genet 2006. PMID:16987884
12: Hou HA, Huang TC, Lin LI et al, WT1 mutation in 470 adult patients with acute myeloid leukemia: stability during disease evolution and implication of its incorporation into a survival scoring system. Blood 2010. PMID:20368469
13: Hou HA, Liu CY, Kuo YY et al, Splicing factor mutations predict poor prognosis in patients with de novo acute myeloid leukemia. Oncotarget 2016. PMID:26812887
14: How J, Sykes J, Gupta V et al, Influence of FLT3-internal tandem duplication allele burden and white blood cell count on the outcome in patients with intermediate-risk karyotype acute myeloid leukemia. Cancer 2012. PMID:22736495
15: Huether R, Dong L, Chen X et al, The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. Nat Commun 2014. PMID:24710217
16: Hyde RK, Liu PP, GATA2 mutations lead to MDS and AML. Nat Genet 2011. PMID:21956389
17: Krauth MT, Alpermann T, Bacher U et al, WT1 mutations are secondary events in AML, show varying frequencies and impact on prognosis between genetic subgroups. Leukemia 2015. PMID:25110071
18: Lauhakirti D, Sritana N, Boonthimat C et al, WT1 mutations and polymorphisms in Southeast Asian acute myeloid leukemia. Exp Mol Pathol 2011. PMID:21798259
19: Lindsley RC, Mar BG, Mazzola E et al, Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood 2015. PMID:25550361
20: Liu Y, He P, Liu F et al, Prognostic significance of NPM1 mutations in acute myeloid leukemia: A meta-analysis. Mol Clin Oncol 2014. PMID:24649346
21: Luo S, Yu K, Yan QX et al, Analysis of WT1 mutations, expression levels and single nucleotide polymorphism rs16754 in de novo non-M3 acute myeloid leukemia. Leuk Lymphoma 2014. PMID:23550990
22: Makishima H, Visconte V, Sakaguchi H et al, Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. Blood 2012. PMID:22323480
23: Micol JB, Duployez N, Boissel N et al, Frequent ASXL2 mutations in acute myeloid leukemia patients with t(8;21)/RUNX1-RUNX1T1 chromosomal translocations. Blood 2014. PMID:24973361
24: Nanaa A, Viswanatha D, Xie Z et al, Clinical and biological characteristics and prognostic impact of somatic GATA2 mutations in myeloid malignancies: a single institution experience. Blood Cancer J 2021. PMID:34193836
25: Niimi K, Kiyoi H, Ishikawa Y et al, GATA2 zinc finger 2 mutation found in acute myeloid leukemia impairs myeloid differentiation. Leuk Res Rep 2013. PMID:24371770
26: Papaemmanuil E, Gerstung M, Bullinger L et al, Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med 2016. PMID:27276561
27: Papaemmanuil E, Gerstung M, Malcovati L et al, Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 2013. PMID:24030381
28: Paschka P, Marcucci G, Ruppert AS et al, Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. J Clin Oncol 2008. PMID:18559874
29: Patel JP, Goenen M, Figueroa ME et al, Prognostic

relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med 2012. PMID:22417203
30: Pratcorona M, Brunet S, Nomdedeu J et al, Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation and concomitant NPM1 mutation: relevance to post-remission therapy. Blood 2013. PMID:23377436
31: Pratz KW, Sato T, Murphy KM et al, FLT3-mutant allelic burden and clinical status are predictive of response to FLT3 inhibitors in AML. Blood 2010. PMID:20007803
32: Rocquain J, Carbuccia N, Trouplin V et al, Combined mutations of ASXL1, CBL, FLT3, IDH1, IDH2, JAK2, KRAS, NPM1, NRAS, RUNX1, TET2 and WT1 genes in myelodysplastic syndromes and acute myeloid leukemias. BMC Cancer 2010. PMID:20678218
33: Small D, FLT3 mutations: biology and treatment. Hematology Am Soc Hematol Educ Program 2006. PMID:17124058
34: Spinner MA, Sanchez LA, Hsu AP et al, GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood 2014. PMID:24227816
35: Thiede C, Steudel C, Mohr B et al, Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood 2002. PMID:12036858
36: Thol F, Kade S, Schlarmann C et al, Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood 2012. PMID:22389253
37: Virappane P, Gale R, Hills R et al, Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. J Clin Oncol 2008. PMID:18591546
38: Vosberg S, Hartmann L, Metzeler KH et al, Relapse of acute myeloid leukemia after allogeneic stem cell transplantation is associated with gain of WT1 alterations and high mutation load. Haematologica 2018. PMID:29954937
39: Yang L, Han Y, Suarez Saiz F et al, A tumor suppressor and oncogene: the WT1 story. Leukemia 2007. PMID:17361230
40: Yoshida K, Sanada M, Shiraishi Y et al, Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 2011. PMID:21909114
41: Zhang SJ, Rampal R, Manshouri T et al, Genetic analysis of patients with leukemic transformation of myeloproliferative neoplasms shows recurrent SRSF2 mutations that are associated with adverse outcome. Blood 2012. PMID:22431577

This result has been reviewed and approved by [redacted]

Low coverage regions:
Listed below are regions where the average sequencing depth (number of times a particular nucleotide is sequenced) in at least 20% of the region-of-interest is less than our stringent cutoff of 300. Sensitivity for detection of low allelic frequency variants may be reduced in areas with reduced depth of coverage.
BCOR(NM_001123385.2) intron 2 exon 2

BACKGROUND INFORMATION: Myeloid Malignancies Mutation and Copy Number Variation Panel by Next Generation Sequencing
'CHARACTERISTICS: Myeloid malignancies are clonal disorders of hematopoietic stem and progenitor cells that include myelodysplastic syndromes (MDSs), myeloproliferative neoplasms (MPNs), myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), acute myeloid leukemia (AML), and others. Recent studies have identified recurrently mutated genes with diagnostic and/or prognostic impact in myeloid malignancies. The presence of certain mutations may inform clinical management. This multigene panel by massively parallel sequencing (next generation sequencing) detects molecular changes (single nucleotide

variants, small insertions and deletions), copy number variants (CNVs) for the targeted genes, and terminal copy number-neutral loss of heterozygosity (CN-LOH). This panel is a more cost-effective approach when compared to the cost of multiple single gene tests and can be used to complement the morphologic and cytogenetic workup of myeloid malignancies.

GENES TESTED: ANKRD26; ASXL1; ASXL2; BCOR; BCORL1; BRAF; CALR; CBL; CBLB; CEBPA; CSF3R; CUX1*; DDX41; DNMT1*; DNMT3A; ELANE; ETNK1; ETV6; EZH2; FBXW7; FLT3; GATA1; GATA2; GNAS; HNRNPK; IDH1; IDH2; IL7R; JAK1; JAK2; JAK3; KDM6A*; KIT; KMT2A; KRAS; LUC7L2; MPL; NOTCH1; NPM1*; NRAS; NSD1; PHF6; PIGA; PPM1D; PRPF40B; PRPF8; PTPN11; RAD21; RUNX1; SAMD9; SAMD9L; SETBP1; SF3B1; SH2B3; SMC1A; SMC3; SRSF2; STAG2; STAT3; STAT5B*; SUZ12*; TET2; TP53; U2AF1; U2AF2; UBA1; WT1; ZRSR2
*One or more exons of the preferred transcript were not covered by sequencing for the indicated gene; see limitations section below.

METHODOLOGY: Genomic DNA was isolated from peripheral blood or bone marrow and then enriched for the targeted exonic regions of the tested genes and approximately 13,000 single nucleotide polymorphisms (SNPs) evenly spaced over the coding genome. The variant status, copy number variation of the targeted genes and SNPs, and CN-LOH were determined by massively parallel sequencing. The hg19 (GRCh37) human genome assembly was used as a reference for identifying genetic variants. The following general types of variants are reported: clinically significant/uncertain sequence variants in the preferred transcript, CNVs (gains or losses) in the targeted genes, likely acquired terminal CN-LOH, and CNVs 5 megabases (Mb) or greater in size in any gene. In addition, these specific variants will also be reported: losses in additional relevant genes (ARID2, ASMTL, ATM, CD200, CDKN2A, CHEK2, ERG, IKZF1, NF1, PAX5, RB1, TBL1XR1), gains in additional relevant genes (MYC), losses between FIP1L1 and PDGFRA that result in a potential fusion, and any CN-LOH involving TP53, JAK2, and CBL.

LIMITATIONS: Variants outside the targeted regions or below the limit of detection are not identified. Variants in regions that are not included in the preferred transcript for the targeted genes are not detected. Benign or likely benign variants and likely germline or interstitial CN-LOH are not reported. In some cases, variants may not be identified due to technical limitations in the presence of pseudogenes or in repetitive or homologous regions. It is also possible some insertion/deletion variants may not be identified. RNA variants, gene fusions, translocations and other structural variants are not detected by this test. Due to complexity of analysis, CNVs may not be reported in the instance of stem cell transplants that present with mixed chimerism, increased genomic complexity (greater than four copy number variants), and complex aneuploidies (e.g., hyper- or hypodiploidy). Variant allele frequency (VAF) is not reported for CNVs with copy number greater than three. This test does not replace conventional cytogenetic studies or genomic microarray in the workup of hematologic malignancies; results from this test should be correlated with cytogenetic test results. Interpretation of this test result may be impacted if this patient has had an undisclosed allogeneic bone marrow transplant or stem cell transplant. This test does not distinguish between somatic and germline variants. The following regions were not sequenced due to technical limitations of the assay:
CUX1 (NM_181552) exon 24
DNMT1 (NM_001130823) exon 5
KDM6A (NM_001291415) exon 13
NPM1 (NM_002520) exon 1
STAT5B (NM_012448) exons 6-9
SUZ12 (NM_015355) exons 1-9

H=High, L=Low, *=Abnormal, C=Critical

LIMIT OF DETECTION (LOD): 5 percent variant allele fraction (VAF) for single nucleotide variants (SNV) and small variants less than 24 base pairs (bp). Variants greater than 24 bp may be detected at LOD, but the analytical sensitivity may be reduced. LOD for CNVs is greater than 2 Mb in size in approximately 30 percent of the sample. LOD for CN-LOH is greater than 10 Mb in approximately 30 percent of the sample. Some areas of the genome may have a reduced sensitivity for CNVs and CN-LOH at LOD.

ANALYTICAL SENSITIVITY: The positive percent agreement (PPA) estimate for the respective variant classes (with 95 percent credibility region) are listed below. Genes included on this test are a subset of a larger methods-based validation from which the PPA values are derived.
Single nucleotide variants (SNVs): 96.9 percent (95.1-98.1 percent)
Insertions/duplications (1-24bp): 98.1 percent (95.5-99.3 percent)
Insertions/duplications (greater than 24bp): > 99 percent (92.9-100.0 percent)
Deletions (1-24bp): 96.7 percent (92.8-98.7 percent)
Deletions (greater than 24bp): 90 percent (79.5-96.1 percent)
Multinucleotide variants (MNVs): 97 percent (93.0-99.0 percent)
FLT3 ITDs: Greater than 99 percent (97.1-100.0 percent)
Copy number gains (greater than 2 Mb): 91.8 percent (86.7-95.3 percent)
Copy number losses (greater than 2 Mb): 92.3 percent (87.7-95.5 percent)
Copy number-neutral loss of heterozygosity (greater than 10 Mb): 98.1 percent (91.5-99.8 percent)

CLINICAL DISCLAIMER: Results of this test must always be interpreted within the context of clinical findings and other relevant data and should not be used alone for a diagnosis or management of malignancy. This test is not intended to detect minimal residual disease.

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the U.S. Food and Drug Administration. This test was performed in a CLIA-certified laboratory and is intended for clinical purposes.

EER Myeloid Mutation Panel NGS, DelDup

See Note

Authorized individuals can access the ARUP Enhanced Report using the following link:

[redacted]

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
MYE CNV Proposed Diagnosis	23-299-108508	10/26/2023 12:21:00 PM	10/26/2023 12:22:11 PM	10/27/2023 8:56:00 AM
MYE CNV Specimen	23-299-108508	10/26/2023 12:21:00 PM	10/26/2023 12:22:11 PM	10/27/2023 8:56:00 AM
MYE CNV Interp	23-299-108508	10/26/2023 12:21:00 PM	10/26/2023 12:22:11 PM	10/27/2023 8:56:00 AM
EER Myeloid Mutation Panel NGS, DelDup	23-299-108508	10/26/2023 12:21:00 PM	10/26/2023 12:22:11 PM	10/27/2023 8:56:00 AM

END OF CHART

H=High, L=Low, *=Abnormal, C=Critical