

Client: Example Client ABC123  
123 Test Drive  
Salt Lake City, UT 84108  
UNITED STATES

Physician: Doctor, Example

**Patient: Patient, Example**

**DOB:** ██████████  
**Gender:** Female  
**Patient Identifiers:** 01234567890ABCD, 012345  
**Visit Number (FIN):** 01234567890ABCD  
**Collection Date:** 00/00/0000 00:00

**Myeloid Malignancies Somatic Mutation and Copy Number Analysis Panel**

ARUP test code 2012182

Myeloid Malignancy Proposed Diagnosis      AML unspec

Myeloid Malignancies Panel Specimen      whole blood

**Myeloid Panel Summary**

See Note

Results Summary:

I. Tier 1 variants detected by next generation sequencing (MYE NGS)

1. CBL c.1259G>A, p.Arg420Gln (NM\_005188.3)  
Variant Frequency: 19.5%

2. TP53 c.743G>A, p.Arg248Gln (NM\_000546.5)  
Variant Frequency: 10.9%

Copy neutral mosaic loss of heterozygosity (CN-LOH) of 4q22.3q35.2 in approximately 47 percent of the sample

**Myeloid Malignancies Panel Interp**

See Note

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

Result:

I. Tier 1 (Variants of known significance in myeloid malignancies):

1. CBL c.1259G>A, p.Arg420Gln (NM\_005188.3)  
Variant Frequency: 19.5%

Interpretation: CBL encodes a RING finger E3 ubiquitin ligase. Somatic mutations of CBL have been reported in 1-33% of patients with acute myeloid leukemia (AML) (1-4), in 9% of patients with secondary AML, and approximately 1% of patients with myelodysplastic syndrome (MDS) (5). They are also seen in 10% of patients with myelodysplastic/myeloproliferative neoplasms (MDS/MPN) (6), including 5-22% of patients with chronic myelomonocytic leukemia (CMML) (5, 7, 8) and 12% of patients with atypical chronic myeloid leukemia (aCML) (7). In addition, germline CBL mutations that predispose patients to

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

juvenile myelomonocytic leukemia (JMML) have also been observed (9, 10). These CBL mutations are often missense substitutions in the RING finger or linker domains that abrogate ubiquitin ligase activity (7). This particular missense mutation occurs in the CBL RING finger domain and has been reported in myeloid malignancies (11). One study concluded that CBL mutations, particularly when homozygous, correlate with shorter overall survival in patients with myeloid malignancies (5). Another study showed patients with CBL mutations have a distinct clinical MDS/MPN phenotype with monocytosis, fibrosis, and splenomegaly (6).

2. TP53 c.743G>A, p.Arg248Gln (NM\_000546.5)  
Variant Frequency: 10.9%

Interpretation: TP53 encodes an important tumor suppressor (p53) that regulates cell cycle progression, apoptosis, DNA repair, and metabolic changes (12). Somatic mutations of TP53 are found in 8% of adult patients with de novo AML (13), in 21-37% of patients with therapy-related myeloid neoplasms, including AML (14, 15), and in 7.5-19% of patients with MDS, mainly in those with advanced diseases (16-18). TP53 mutations are also found in 1-5% of patients with CMML (19). This particular missense mutation occurs in the DNA-binding domain and is predicted to inactivate the tumor suppressor p53 (20). In adult AML patients, TP53 mutations are commonly associated with a complex karyotype and are identified as an unfavorable factor for achieving complete remission (21-23). In AML patients with both complex karyotype and TP53 mutations, outcome is quite poor (22, 24). Mutated TP53 is associated with a complex karyotype and shorter overall survival in patients with therapy-related myeloid neoplasms (14, 15). In MDS patients, TP53 mutations strongly correlate with complex karyotype, aberrations of chromosome 5, high risk of leukemic transformation, and poor overall survival (16, 17, 25). Mutated TP53 also predicts shorter overall survival in MDS patients after hematopoietic stem-cell transplantation (18, 26). One study found that TP53 mutations with variant allele frequencies higher than 40% strongly predict for complex cytogenetics and poor prognosis in patients with MDS (27). In MPN patients, TP53 mutations are associated with progression to AML and decreased overall survival (28, 29).

II. Tier 2 (Variants of unknown significance in myeloid malignancies):

1. ETNK1 c.730A>G, p.Asn244Asp (NM\_018638.4)  
Variant Frequency: 4.6%

Interpretation: ETNK1 encodes an ethanolamine kinase, which catalyzes the first step of the de novo phosphatidylethanolamine (PE) biosynthesis pathway. Somatic mutations of ETNK1 are identified in 2% of MDS patients or AML patients secondary to MDS (30). Somatic mutations of ETNK1 are identified in 3-14% of patients with CMML, 9% of patients with aCML, and 6% of patients with systemic mastocytosis (31, 32). This particular missense variant alters a highly conserved amino acid and has not been reported in myeloid malignancies, to the best of our knowledge. Its functional consequences are unknown. The clinical significance, if any, is uncertain. Please note that the variant allele frequency is below 5.0%.

#### References:

- 1.R. Ghassemifar et al., Incidence of c-Cbl mutations in human acute myeloid leukaemias in an Australian patient cohort. Pathology 2011. PMID: 21436637.
- 2.S. Abbas et al., Exon 8 splice site mutations in the gene encoding the E3-ligase CBL are associated with core binding factor acute myeloid leukemias. Haematologica 2008. PMID: 18698078.

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- 3.C. Reindl et al., CBL exon 8/9 mutants activate the FLT3 pathway and cluster in core binding factor/11q deletion acute myeloid leukemia/myelodysplastic syndrome subtypes. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2009. PMID: 19276253.
- 4.M. S. Fernandes et al., Novel oncogenic mutations of CBL in human acute myeloid leukemia that activate growth and survival pathways depend on increased metabolism. *The Journal of biological chemistry* 2010. PMID: 20622007.
- 5.H. Makishima et al., Mutations of e3 ubiquitin ligase cbl family members constitute a novel common pathogenic lesion in myeloid malignancies. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009. PMID: 19901108.
- 6.J. Schwaab et al., Activating CBL mutations are associated with a distinct MDS/MPN phenotype. *Annals of hematology* 2012. PMID: 23010802.
- 7.F. H. Grand et al., Frequent CBL mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. *Blood* 2009. PMID: 19387008.
- 8.A. Kohlmann et al., Next-generation sequencing technology reveals a characteristic pattern of molecular mutations in 72.8% of chronic myelomonocytic leukemia by detecting frequent alterations in TET2, CBL, RAS, and RUNX1. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010. PMID: 20644105.
- 9.M. L. Loh et al., Mutations in CBL occur frequently in juvenile myelomonocytic leukemia. *Blood* 2009. PMID: 19571318.
- 10.B. Perez et al., Germline mutations of the CBL gene define a new genetic syndrome with predisposition to juvenile myelomonocytic leukaemia. *Journal of medical genetics* 2010. PMID: 20543203.
- 11.COSMIC database website:  
<http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/>
- 12.R. Beckerman, C. Prives, Transcriptional regulation by p53. *Cold Spring Harb Perspect Biol* 2010. PMID: 20679336.
- 13.N. Cancer Genome Atlas Research, Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N. Engl. J. Med.* 2013. PMID: 23634996.
- 14.A. H. Shih et al., Mutational analysis of therapy-related myelodysplastic syndromes and acute myelogenous leukemia. *Haematologica* 2013. PMID: 23349305.
- 15.R. C. Lindsley et al., Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood* 2015. PMID: 25550361.
- 16.A. G. Kulasekararaj et al., TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. *British journal of haematology* 2013. PMID: 23297687.
- 17.R. Bejar et al., Clinical effect of point mutations in myelodysplastic syndromes. *The New England journal of medicine* 2011. PMID: 21714648.
- 18.R. C. Lindsley et al., Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation. *The New England journal of medicine* 2017. PMID: 28177873.
- 19.M. M. Patnaik et al., Prognostic interaction between ASXL1 and TET2 mutations in chronic myelomonocytic leukemia. *Blood cancer journal* 2016. PMID: 26771811.
- 20.IARC TP53 database website:  
<http://p53.iarc.fr/TP53GeneVariations.aspx>
- 21.R. Kihara et al., Comprehensive analysis of genetic alterations and their prognostic impacts in adult acute myeloid leukemia patients. *Leukemia* 2014. PMID: 24487413.
- 22.E. Papaemmanuil et al., Genomic Classification and Prognosis in Acute Myeloid Leukemia. *The New England journal of medicine* 2016. PMID: 27276561.
- 23.C. Y. Ok et al., Mutational profiling of therapy-related myelodysplastic syndromes and acute myeloid leukemia by next generation sequencing, a comparison with de novo diseases. *Leukemia research* 2015. PMID: 25573287.
- 24.F. G. Rucker et al., TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy

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number alterations, monosomal karyotype, and dismal outcome. Blood 2012. PMID: 22186996.

25.M. Cazzola et al., The genetic basis of myelodysplasia and its clinical relevance. Blood 2013. PMID: 24136165.

26.R. Bejar et al., Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014. PMID: 25092778.

27.D. A. Sallman et al., Impact of TP53 mutation variant allele frequency on phenotype and outcomes in myelodysplastic syndromes. Leukemia 2015. PMID: 26514544.

28.A. Harutyunyan et al., p53 lesions in leukemic transformation. The New England journal of medicine 2011. PMID: 21288114.

29.P. Lundberg et al., Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood 2014. PMID: 24478400.

30.M. G. Della Porta et al., Clinical Effects of Driver Somatic Mutations on the Outcomes of Patients with Myelodysplastic Syndromes Treated with Allogeneic Hematopoietic Stem-Cell Transplantation. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2016. PMID: 27601546.

31.C. B. Gambacorti-Passerini et al., Recurrent ETNK1 mutations in atypical chronic myeloid leukemia. Blood 2015. PMID: 25343957.

32.T. L. Lasho et al., Novel recurrent mutations in eethanolamine kinase 1 (ETNK1) gene in systemic mastocytosis with eosinophilia and chronic myelomonocytic leukemia. Blood cancer journal 2015. PMID: 25615281.

#### Low coverage regions:

This list contains exons where the average sequencing depth (number of times a particular position is sequenced) is below our stringent cutoff of 300. The sequencing reads from these exons were manually reviewed. If high quality variants are detected in these regions they will be listed above in Tier 1 or Tier 2.

NONE

This result has been reviewed and approved by Jay Patel, M.D.

#### BACKGROUND INFORMATION: Myeloid Malignancies Panel Interp

CHARACTERISTICS: Myeloid malignancies are clonal disorders of hematopoietic stem and progenitor cells that include myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), myelodysplastic/myeloproliferative neoplasms (MDS/MPN), and acute myeloid leukemia (AML). 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 multi-gene panel by massively parallel sequencing (next generation sequencing) is a more cost-effective approach when compared to the cost of multiple single gene tests. This test 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, PRPF40B, PRPF8, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5B\*, SUZ12\*, TET2, TP53, U2AF1, U2AF2, 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

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

bone marrow and then enriched for the targeted exonic regions of the tested genes. The variant status of the targeted genes was determined by massively parallel sequencing. The hg19 (GRCh37) human genome assembly was used as a reference for identifying genetic variants.

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. 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. 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

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 24bp may be detected at LOD, but the analytical sensitivity may be reduced.

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)

Multi-nucleotide variants (MNVs): 97 percent (93.0 - 99.0 percent)

FLT3 ITDs: Greater than 99 percent (97.1 - 100.0 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 of malignancy. This test is not intended to detect minimal residual disease.

Test developed and characteristics determined by ARUP Laboratories. See Compliance Statement B: aruplab.com/CS

## Cytogenomic Microarray SNP - Oncology

**Abnormal** \* (Ref Interval: Normal)

CMA ONC TEST RESULT

Copy neutral mosaic loss of heterozygosity (CN-LOH) of 4q22.3q35.2 in approximately 47 percent of the sample

Sex chromosome complement: XY (male)

Interpretation:

CN-LOH is a common finding in hematological neoplasms. The proposed mechanisms for CN-LOH in carcinogenesis include unmasking mutations of tumor suppressor genes, as well as providing a selective advantage to cells harboring gain-of-function mutations in some proto-oncogenes. 4q24 copy neutral loss of heterozygosity (CN-LOH) associated with TET2 mutations has been reported in myeloid disorders, including AML, MDS and MPN. Please correlate this result with clinical and other laboratory findings.

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

Correlation with other clinical findings is recommended.

References:

- 1) Gondek et al. Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, and MDS-derived AML. *Blood* 2008; 111(3):1534-42.
- 2) O'Keefe et al. Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies. *Blood* 2010; 115(14):2731-9.
- 3) Makishima H and Maciejewski JP. Pathogenesis and consequences of uniparental disomy in cancer. *Clin Cancer Res* 2011; 17(12):3913-23.
- 4) Mohamedali et al. Novel TET2 mutations associated with UPD4q24 in myelodysplastic syndrome. *J Clin Oncol*. 2009; 27(24):4002-6.
- 5) Jankowska et al. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. *Blood* 2009; 113(25):6403-10.

No other significant DNA copy number changes or copy neutral long contiguous stretches of homozygosity were detected.

SUMMARY OF ABNORMALITIES DETECTED (PATHOGENIC, ACQUIRED):  
arr[GRCh37] 4q22.3q35.2(97647903\_190921709)x2 mos hnz

Test Information:

Chromosomal microarray analysis (CMA) was performed using Affymetrix CytoScan HD microarray. This microarray consists of 2,696,550 oligonucleotide probes across the genome, including 1,953,246 unique non-polymorphic probes, and 743,304 SNP (single nucleotide polymorphism) probes. Patient hybridization parameters are normalized to a reference set derived from 100 individuals with normal microarray results. Genomic linear positions are given relative to NCBI build 37 (hg19). Detected aberrations are reported when found to have clear or suspected clinical relevance; aberrations devoid of relevant gene content or reported as common findings in the general population may not be reported.

This microarray and associated software (Chromosome Analysis Suite) are manufactured by Affymetrix and used by ARUP Laboratories for the purpose of identifying DNA copy number gains and losses associated with large chromosomal imbalances. This analysis will not detect all forms of polyploidy, balanced rearrangements (eg. inversions and balanced chromosomal translocations), small deletions, point mutations, and some mosaic conditions. While this assay has been extensively validated by ARUP Laboratories and other clinical laboratories per ACMG guidelines, it is not feasible to validate every potential genomic imbalance in the human genome. Furthermore, this technique only identifies the regions of imbalance; it does not provide information regarding the arrangement or mechanisms responsible. For these reasons, we may recommend that some chromosomal microarray results be characterized by fluorescence in situ hybridization (FISH) or standard chromosome analysis.

The functional resolution of this assay varies across different samples dependent upon the size of the abnormality, probe density, tumor content and quality of the DNA obtained. On average, the limit of detection will vary from less than 100 kilobases for samples with high tumor content (generally greater than 70 percent) to several megabases for samples with lower tumor content (25-35 percent). The limit of detection for loss of heterozygosity (LOH) is approximately 3 megabases.

This result has been reviewed and approved by Erica F. Andersen, Ph.D., FACMG

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INTERPRETIVE INFORMATION: Cytogenomic Microarray  
SNP - Oncology  
Test developed and characteristics determined by ARUP  
Laboratories. See Compliance Statement B: aruplab.com/CS

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Myeloid Malignancy Proposed Diagnosis	20-015-111092	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Myeloid Malignancies Panel Specimen	20-015-111092	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Myeloid Panel Summary	20-015-111092	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Myeloid Malignancies Panel Interp	20-015-111092	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Cytogenomic Microarray SNP - Oncology	20-015-111092	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

END OF CHART

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