NGS for Myeloid Neoplasm (MNGS)

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Warde Medical Laboratory

Powered By Pierian

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
Test Patient1	01/01/2000	Myeloid neoplasm	0000011		Final

REPORT SUMMARY

Executive Summary

Three mutations of potential clinical importance are detected (see details below).

Copy number loss for genes CDC25, DDX41, and RPS14 are consistent with 5q-/5-.

Reviewed by Matthew Sekedat, Ph.D.

Genomic Findings

IA		IB	IIC	IID
ASXL1	p.G645Wfs*13 c.1932_1933insT	No variants reported.	No variants reported.	No variants reported.
ETV6	p.L201P c.602T>C			
TP53	p.H179R c.536A>G			

CLINICALLY RELEVANT RESULTS

Tier I - Strong Clinical Significance

VARIANT	CLINICAL IMPACT
ASXL1 p.G645Wfs*13 c.1932_1933insT A NM_015338.5 VAF % 33.6 DEPTH 2,232	**Unfavorable Prognosis in** — Aggressive systemic mastocytosis, Indolent systemic mastocytosis, Polycythemia vera (clinical), Polycythemia vera, Chronic myeloid leukemia, Acute myeloid leukemia, disease, Myelodysplastic syndrome, Systemic mastocytosis with associated clonal, hematologic non-mast-cell lineage disease, Mast cell leukemia (clinical), Chronic myelomonocytic leukemia, Myelosclerosis with myeloid metaplasia, Acute myeloid leukemia, or Myeloproliferative neoplasm INTERPRETATION ASXL1 is a member of the polycomb group of proteins which are necessary for the maintenance of stable repression of homeotic and other loci resulting in enhanced transcription of certain genes while repression of transcription of other genes (RefSeq, Sep 2009).

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VARIANT	CLINICAL IMPACT

INTERPRETATION

Myelodysplastic syndromes (MDS):

Classification: Tier IA

In MDS, ASXL1 mutations (typically nonsense and frameshift mutations) are independently associated with a poor prognosis (NCCN, MDS v3.2023).

In MDS patients, mutations in ASXL1 were found to be associated with significant negative overall response rate on treatment with azacitidine and decitabine, however, had no impact OS (PMID 31312376). In MDS patients, mutations in ASXL1 were found to be associated with inferior complete remission rate on treatment with azacitidine, decitabine, or azacitidine in combination with an additional agent, however, were reported to have no impact on OS and overall response rate (PMID 33591325). In primary MDS patients, mutations in ASXL1 were reported to adversely affect response to hypomethylating agents and lenalidomide but did not impact response to erythropoiesis-stimulating agents (PMID 30152885).

ASXL1 mutations were observed to have no impact on survival in MDS and MDS/MPN patients undergoing a conditioning regimen of 5-day decitabine administration for allo-HSCT (PMID 31494229).

Myeloproliferative Neoplasms (MPN):

Classification: Tier IA

For pre-primary myelofibrosis (PMF) and overt PMF, in absence of the 3 major clonal mutations (JAK2, MPL, and CALR), the presence of another clonal markers (including ASXL1) is one of the major diagnostic criteria and can be useful to determine the clonal nature of the disease (NCCN, MPN v1.2024).

In PMF, ASXL1 mutations are considered as 'high-molecular risk' (HMR) mutations and are associated with an inferior OS and leukemia free survival (LFS) independent of IPSS or DIPSS-Plus risk score (NCCN, MPN v1.2024).

In MF patients, ASXL1 mutations were associated with inferior LFS following hematopoietic cell transplantation (HCT) (NCCN, MPN v1.2024).

In polycythemia vera (PV), the presence of adverse variants/mutations (including ASXL1) is associated with inferior overall survival, affected myelofibrosis-free survival but it did not significantly affect the LFS (NCCN, MPN v1.2024).

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VARIANT | CLINICAL IMPACT

INTERPRETATION

Acute myeloid leukaemia (AML):

Classification: Tier IA

ASXL1 mutations in de novo acute myeloid leukaemia (AML) cases, are associated with poor outcomes. ASXL1 mutations is also identified as one of the genetic abnormalities associated with a poor/adverse risk subgroup in non-APL AML patients, except when co-occurring with favorable risk AML subtypes (NCCN, AML v6.2023).

In untreated t-AML or MRC-AML patients, high-risk molecular prognosis subgroups defined by 2017 ELN risk stratification (including ASXL1 mutations) were not reported to impact response rate of CPX-351 (liposomal formulation of cytarabine and daunorubicin) (PMID 33570629).

In AML patients treated with venetoclax in combination with azacitidine, ASXL1 mutations had negative impact on progression free survival (PMID 38095287).

The impact of ASXL1 mutation in AML patients undergoing allogeneic stem cell transplantation (allo-HSCT) is unclear (PMID 33840380 (may not benefit); 29321554 (no impact)).

Chronic myelomonocytic leukaemia (CMML)

Classification: Tier IA

ASXL1 mutations (typically nonsense and frameshift) are independently associated with a poor prognosis in chronic myelomonocytic leukaemia (CMML) (NCCN, MDS v3.2023).

In CMML patients treated with hypomethylating agents (azacitidine or decitabine), the impact of ASXL1 mutation on overall survival (OS) or response is unclear (PMID 29728305 (may not benefit); PMID 30964202; 31377458 (No impact)).

Multiple studies with CMML patients undergoing allo HCT, reported that ASXL1 mutations showed no impact on prognosis (PMID 31289199; 34500124; 33219206; 35443559).

ETV6

p.L201P
c.602T>C

A

NM_001987.4
VAF % 50.2

INTERPRETATION

Note: Based on VAF, this could be a germline alteration

ETV6 is a transcriptional repressor that plays a role in hematopoiesis and malignant transformation (PMID: 25581430, 2015).

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VARIANT | CLINICAL IMPACT

DEPTH 872

INTERPRETATION

Myelodysplastic Syndromes (MDS):

Classification: Tier IA

ETV6 mutations (typically nonsense and frameshift mutations) are independently associated with a poor prognosis (NCCN, MDS v3.2023).

ETV6 mutations were found to have no impact on survival in MDS patients treated with allogeneic hematopoietic cell transplantation (HCT) with or without a conditioning regimen of 5-day decitabine (PMID 31494229, 28971906).

TP53

p.H179R
c.536A>G

A

NM_000546.5
VAF % 52.6
DEPTH 496

Unfavorable Prognosis in

— Essential thrombocythemia, Medulloblastoma, Myelosclerosis with myeloid metaplasia, Myeloproliferative disorder, or Myeloproliferative neoplasm

INTERPRETATION

A missense mutation, p.H179R, was identified in TP53. This specific mutation resides in the P53 DNA-binding domain (95-288), results in a loss-of-function, and is likely to be oncogenic (PMID: 28481359). This specific mutation has also been reported by ClinVar, both germline and somatic, as "pathogenic/likely pathogenic(23)" in various syndromes and malignancies, including acute myeloid leukemia (AML) (somatic, likely pathogenic) (Variation ID: 376606). The NCCN guidelines list TP53 mutations are associated with an unfavorable risk and poor outcomes (NCCN guidelines, AML).

TP53 encodes the p53 tumor suppressor protein, a transcription factor that responds to cellular stresses, including DNA damage and oncogenic activation, by inducing downstream anti-tumor responses such as DNA repair and apoptosis (PMID: 11099028, 28481359). TP53 is one of the most commonly mutated genes in cancer and have been reported in approximately 7% of AML cases (cBioPortal, December 2023). TP53 variants are often found to be associated with an unfavorable prognosis. TP53 mutations were found to be associated with poor survival in patients with AML (PMID: 22887079, 15084693). However, it has been reported that AML and MDS with TP53 mutation have a better response rate to Decitabine (N Engl J Med 2016;375:2023-36). It has been reported TP53 mutation appears to predict poorer response to lenalidomide in patients with del(5q) (PMID: 23614682, 24682512, 21519010). Germline variants of TP53 have been associated with Li-Fraumeni syndrome. Clinical investigation is underway to evaluate targeted therapies in TP53-mutated cancers (PMID: 19043449, 24651012, 22101337). Clinical correlation is recommended.

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Tier II - Potential Clinical Significance

No variants were reported for this classification tier.

FREE TEXT

TIER III - VARIANTS OF UNCERTAIN SIGNIFICANCE

ASXL1
p.G704R
NM_015338.5
c.2110G>A
VAF 48.3 %
DEPTH 1,144

CDC25C
Copy number loss in *CDC25C* (1 copy)

DDX41
Copy number loss in *DDX41* (1 copy)

RPS14
Copy number loss in *RPS14* (1 copy)

SH2B3
p.V462M
NM_005475.2
c.1384G>A
VAF 46.4 %
DEPTH 563

CLASSIFICATION AND LEVELS OF EVIDENCE

The variant classification system used in this report is based on joint consensus recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and the College of American Pathologists (J Mol Diagn 2017, 19:4-23). Tiers IA, IB, IIC, IID, III and IV describe variant categories of descending clinical significance in the patient. Variants in Tier IV are not reported in accordance with the consensus recommendations.

IA	IB	IIC	IID
Variant of strong clinical significance, Level A evidence (FDA approved therapy or practice guideline in patient's tumor type)	Variant of strong clinical significance, Level B Evidence (consensus in the field based on well-powered studies in patient's tumor type)	Variant of potential clinical significance, Level C evidence (FDA approved therapy or practice guideline in other tumor type(s), evidence from multiple small published studies, or based on availability of investigational therapies)	Variant of potential clinical significance, Level D evidence (case reports or preclinical studies)

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III	Variant of uncertain clinical significance
IV	Benign or likely benign variant

TEST DETAILS

REPORTED GENES

ABL1, *ANKRD26*, *ASXL1*, *ATRX*, *BCOR*, *BCORL1*, *BRAF*, *BTK*, *CALR*, *CBL*, *CBLB*, *CBLC*, *CCND2*, *CDC25C*, *CDKN2A*, *CEBPA*, *CSF3R*, *CUX1*, *CXCR4*, *DCK*, *DDX41*, *DHX15*, *DNMT3A*, *ETNK1*, *ETV6*, *EZH2*, *FBXW7*, *FLT3*, *GATA1*, *GATA2*, *GNAS*, *HRAS*, *IDH1*, *IDH2*, *IKZF1*, *JAK2*, *JAK3*, *KDM6A*, *KIT*, *KMT2A*, *KRAS*, *LUC7L2*, *MAP2K1*, *MPL*, *MYC*, *MYD88*, *NF1*, *NOTCH1*, *NPM1*, *NRAS*, *PDGFRA*, *PHF6*, *PPM1D*, *PTEN*, *PTPN11*, *RAD21*, *RBBP6*, *RPS14*, *RUNX1*, *SETBP1*, *SF3B1*, *SH2B3*, *SLC29A1*, *SMC1A*, *SMC3*, *SRSF2*, *STAG2*, *STAT3*, *TET2*, *TP53*, *U2AF1*, *U2AF2*, *WT1*, *XPO1*, *ZRSR2*

CGW VERSION

CGW_v6.26

DATABASE DETAILS

The versions, releases, builds, dates of the following databases were used to generate this report.

- Genomic Build: GRCh37.p13
- Genomic Annotation Sources: NCBI RefSeq v105
- gnomAD: r2.1
- dbscSNV: v1.1
- COSMIC: v98
- dbSNP: 149
- NHLBI ESP: v.0.0.30
- ClinVar: 20230403
- dbNSFP: 4.3c
- ExAC: v1.0

CODING EXON COVERAGE METRICS

Level 2: 200x coverage for > 95% of positions was not achieved for the targeted exon regions listed below:

Gene	Transcript ID (Exon/Intron('))
IDH1	NM_005896.2 (3)
PHF6	NM_032335.3 (4)
PTPN11	NM_002834.3 (7)

METHODOLOGY

Assay Methods: Warde Medical Laboratory's MNGS test is a targeted next-generation sequencing (NGS) assay designed to identify genomic alterations in whole blood and bone marrow samples. The MNGS test uses Anchored-Multiplex PCR enrichment chemistry (Integrated DNA Technology) and is designed to detect multiple classes of variants including single nucleotide variants (SNVs), small insertions/deletions (indels), internal tandem duplications (ITDs), and certain copy number variations (CNVs) in selected genes relevant to Myeloid Neoplasms.

DNA specimens are extracted from a whole blood or bone marrow. Preparation of sequencing libraries includes end repair of enzymatically

fragmented DNA, annealing of universal adaptors with sample indices and molecular barcodes, and target enrichment with VariantPlex gene-specific primers. Denatured library pools are sequenced using Illumina v2 or v3 reagents on a MiSeq with a minimum target depth of 3 million reads per sample.

Bioinformatic Analysis Methods: Sequencing files are uploaded to Archer Analysis Unlimited (AAU) Platform v7.1 for identification of genomic alterations according to the human genome reference build GRCh37 (hg19). Variant classification files are then uploaded to Clinical Genomic Workspace (Velsera) for variant classification and report generation. Variants are reported according to HGVS nomenclature (hgvs-nomenclature.org) and classified according to the AMP classification system into tiers IA, IB, IIC, IID, III and IV. These tiers are stratified by clinical utility ('actionability' for clinical decision-making as to diagnosis, prognosis, treatment options, and carrier status) and previously reported data in the medical literature. Variations found in gnomAD (https://gnomad.broadinstitute.org/) that have 1% minor allele frequency (except those that are also in Clinvar denoted as clinically relevant, used in a clinical diagnostic assays, or reported as a mutation in a publication) are classified as tier IV benign variants. Tier IV variants are not included in the report.

Test Performance: Single base substitutions, small indels, and small duplications (up to 100 bp): accuracy >99%; reproducibility >99%; sensitivity ≥5% variant allele fraction (VAF) with coverage ≥200x. Larger indels (101 bp to 1 kb) will be reported if observed with a VAF ≥5% and coverage ≥200x. Events larger than 1 kb such as gene-level copy number variants (CNVs) may be identified and gain/loss will be reported when detected at coverage ≥200x.

Additional Notes:

- Copy Number Variation (CNV) is assessed using a group of CNV-normal control samples as a baseline.
- This assay is clinically validated for the detection of somatic variants in samples of myeloid-origin malignancies.
- It is possible that pathogenic variants may not be reported by one or more of the tools because of the parameters used. However, tool parameters were optimized to maximize specificity and sensitivity.

DISCLAIMER

This Report was generated using the materials and methods described above, which required the use of various reagents, protocols, instruments, software, databases, and other items, some of which were provided or made accessible by third parties. A defect or malfunction in any such reagents, protocols, instruments, software, databases, and/or other items may compromise the quality or accuracy of the Report.

The Report has been created based on, or incorporates references to, various scientific manuscripts, references, and other sources of information, including without limitation manuscripts, references, and other sources of information that were prepared by third parties that describe correlations between certain genetic mutations and particular diseases (and/or certain therapeutics that may be useful in ameliorating the effects of such diseases). Such information and correlations are subject to change over time in response to future scientific and medical findings. **Warde Medical Laboratory** makes every effort to monitor the information used to generate a report and will issue a corrected report when information changes.

The Report must always be interpreted within the clinical context. A physician should always consider the Report along with all other pertinent information and data prior to providing a diagnosis to a patient or developing and implementing a plan of care for a patient. The Report should never be considered or relied upon alone in making any diagnosis or prognosis. The manifestation of many diseases are caused by more than one gene variant, a single gene variant may be relevant to more than one disease, and certain relevant gene variants may not have been considered in the Report. In addition, many diseases are caused or influenced by modifier genes, epigenetic factors, environmental factors, and other variables that are not addressed by the Report (or that are otherwise unknown). This Report is based on an NGS assay which does not distinguish between somatic and germline variants. As such, the relevance of the Report should be interpreted in the context of a patient's clinical manifestations.

The test performance characteristics were determined by **Warde Medical Laboratory**. The Report was generated by the **Warde Medical Laboratory** as required by the CLIA 1988 regulations. The Report, and the tests used to generate the Report, have not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. The test results have been shown to be clinically useful. This laboratory is CLIA certified to perform high complexity testing.

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REFERENCES

PMID **11099028**: (Vogelstein B, Lane D, Levine AJ; Surfing the p53 network.; Nature; 2000 Nov 16;408(6810):307-10)

PMID **19043449**: (Vazquez A, Bond EE, Levine AJ, Bond GL; The genetics of the p53 pathway, apoptosis and cancer therapy.; Nat Rev Drug Discov; 2008 Dec;7(12):979-87)

PMID **22887079**: (Milosevic JD, *et al.*; Clinical significance of genetic aberrations in secondary acute myeloid leukemia.; Am J Hematol; 2012 Nov;87(11):1010-6)

PMID **23614682**: (Mallo M, *et al.*; Response to lenalidomide in myelodysplastic syndromes with del(5q): influence of cytogenetics and mutations.; Br J Haematol; 2013 Jul;162(1):74-86)

PMID **25581430**: (Zhang MY, *et al.*; Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy.; Nat Genet; 2015 Feb;47(2):180-5)

PMID **28481359**: (Zehir A, *et al.*; Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients.; Nat Med; 2017 Jun;23(6):703-713)

PATIENT AND ORDER DETAILS

PATIENT

DATE OF BIRTH
01/01/2000
SEX

PHYSICIAN

FACILITY
Warde Lab
ORDERING PHYSICIAN
Kajal Sitwala

SPECIMEN

SPECIMEN TYPE
Blood specimen
EXT. SPECIMEN ID
DATE COLLECTED
02/12/2024
DATE RECEIVED
02/12/2024

CASE

DATE REPORTED