NGS for Myeloid Neoplasm (MNGS)

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Warde Medical Laboratory

Powered By Pierian

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
		Myeloid neoplasm			Final

REPORT SUMMARY

Executive Summary

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

Note low (2.9%) variant allele frequency for the reported ASXL1 mutation, as compared to 40.3% variant allele frequency for NRAS mutation. This suggests that ASXL1 mutation was present in only a small minority (<10%) of neoplastic cells from the submitted specimen, so its clinical significance at this time is uncertain.

NRAS mutation carries greater clinical significance in MDS/MPN than in AML; interpret with caution.

Reviewed by Matt Sekedat, Ph.D. and Kajal Sitwala, MD, Ph.D.

Genomic Findings

IA		IB	IIC	IID
ASXL1	p.G646Wfs*12 c.1934dupG	No variants reported.	No variants reported.	No variants reported.
NRAS	p.Q61L c.182A>T			

CLINICALLY RELEVANT RESULTS

Tier I - Strong Clinical Significance

VARIANT	CLINICAL IMPACT
ASXL1 p.G646Wfs*12 c.1934dupG A NM_015338.5 VAF % 2.9 DEPTH 2,411	**Unfavorable Prognosis in** — Myeloproliferative neoplasm, Chronic myeloid leukemia, Chronic myelomonocytic leukemia, Myelodysplastic syndrome, Acute myeloid leukemia, or Acute myeloid leukemia, disease 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).

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
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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).

Studies show that AML patients with ASXL1 mutations may not benefit from allogeneic stem cell transplantation (allo-HSCT) (PMID 33840380; https://doi.org/10.1016/j.jtct.2023.12.164 (may not benefit); 29321554 (no impact)).

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):

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
		Myeloid neoplasm			Final

VARIANT | CLINICAL IMPACT

INTERPRETATION

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).

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).

Clinical trial: NCT04734990 - phase I/II

NRAS
p.Q61L
c.182A>T

A

Unfavorable Prognosis in

— Chronic myelomonocytic leukemia, Myelodysplastic syndrome, or Myeloproliferative neoplasm

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
		Myeloid neoplasm			Final

VARIANT

NM_002524.4
VAF % 40.3
DEPTH 747

CLINICAL IMPACT

INTERPRETATION

The NRAS protein is a small GTPase that is important in the control of proliferation in the RAS-MAPK growth signaling pathway. MutatioNRAS encodes a membrane protein with GTPase activity that can activate the PI3K and MAPK pathways involved in cell survival, growth, differentiation, and proliferation (PMID: 26322273, 2015; 25252692, 2014).

Acute Myeloid Leukemia (AML)

Classification: Tier IIC

In a retrospective study with AML patients post-hematopoietic stem cell transplantation, patients carrying NRAS mutation showed a higher incidence of relapse as compared to the non-mutated group (p=0.05) (PMID 36568206). In AML patients post allo-HSCT, NRAS mutation was significantly associated with worse OS (Ref https://doi.org/10.1016/j.jtct.2023.12.164).

In patients with relapsed or refractory AML (RR-AML) treated with venetoclax in combination with azacitidine/decitabine/low-dose cytarabine, mutations in NRAS were associated with worse OS (PMID 33687434).

The therapeutic impact of NRAS mutations is unclear in patients with acute myeloid leukemia treated with chemotherapy (PMID 33650111, 34094982 (no impact); 32796597 (may not benefit: in patients lacking both FLT3-ITD and NPM1).

Myelodysplastic Syndrome (MDS)

Classification: Tier IA

In MDS, NRAS missense mutations (at codons 12, 13, 61) are associated with a poor prognosis, particularly in patients predicted to have lower-risk MDS (NCCN, MDS v3.2023).

In MDS patients, NRAS mutations were associated with the highest CR/PR (complete remission/partial remission) rates to azacitidine treatment (PMID 29963245).

In MDS patients who underwent hematopoietic stem cell transplantation, patients with NRAS mutations we associated with worse OS (PMID 32798413, 38232336).

Chronic Myelomonocytic Leukemia (CMML)

Classification: Tier IA

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
		Myeloid neoplasm			Final

VARIANT	CLINICAL IMPACT
	INTERPRETATION

In CMML, NRAS missense mutations (at codons 12, 13, 61) are associated with a poor prognosis (NCCN, MDS v3.2023).

Studies show that CMML patients with NRAS mutations may not benefit from allogeneic stem cell transplantation (allo-HSCT) (PMID 33755092, 32513965, 34500124, 31289199 (may not benefit); 35443559 (no impact)).

In CMML patients treated with hypomethylating agents (HMA), mutations in RAS had no effect on overall survival (PMID 29728305).

Myeloproliferative Neoplasms (MPN):

Classification: Tier IA

In primary myelofibrosis, mutations in RAS were associated with decreased overall survival (NCCN, MPN v1.2024).

The therapeutic impact of RAS is unfavourable in patients with myelofibrosis treated with JAK inhibitor (PMID 33197049). In blast phase MPN patients, treated with hypomethylating agent (HMA) and venetoclax, N/KRAS mutations were significantly associated with lower CR/CRi rate (PMID 33844862).

Clinical trial: NCT04734990 - phase I/II

Tier II - Potential Clinical Significance

No variants were reported for this classification tier.

FREE TEXT

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
		Myeloid neoplasm			Final

TIER III - VARIANTS OF UNCERTAIN SIGNIFICANCE

No variants were reported for this classification tier.

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)

III	Variant of uncertain clinical significance

IV	Benign or likely benign variant

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
		Myeloid neoplasm			Final

PERTINENT NEGATIVES

Pertinent negatives were not reported for this case.

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
- NHLBI ESP: v.0.0.30
- dbscSNV: v1.1
- ClinVar: 20230403
- dbNSFP: 4.3c
- ExAC: v1.0
- dbSNP: 149
- gnomAD: r2.1
- COSMIC: v98

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('))			
HRAS NM_005343.2 (4)	***IDH1*** NM_005896.2 (3)	***PTPN11*** NM_002834.3 (7)	***TP53*** NM_000546.5 (10)

METHODOLOGY

Assay Methods: This test utilized the Archer® VariantPlex® Myeloid targeted next-generation sequencing assay to detect DNA based variants in blood and bone marrow samples. The Archer® VariantPlex® Myeloid assay utilized Anchored-Multiplex PCR (AMPTM) enrichment chemistry (Archer/IDT), allowing greater read de-duplication and error correction prior to downstream analysis. The Archer VariantPlex® assay was designed to detect multiple classes of variants including single nucleotide variants (SNVs), small Insertions /Deletions (Indels), some large deletions, copy number variations (CNVs), and internal tandem duplications (ITDs) in selected genes of clinical relevance.

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
ALEXANDRIA GEORGE	12/10/1992	Myeloid neoplasm	TH101893725	02/12/2024	Final

Library preparation, using a normalized DNA input concentration, consisted of DNA fragmentation, end repair, annealing of universal adaptors with sample indices and molecular barcodes, target enrichment with VariantPlex unidirectional gene-specific primers, and measurement by KAPA Universal Library Quantification Kit for standardization in library pooling. Sequencing of denatured library pools by Illumina v2 or v3 reagents was performed on the MiSeq instrument with minimum reads targeted at 6.5 million reads per sample. Pooled DNA libraries (both DNA and RNA) were then denatured and sequenced using Illumina v2 or v3 reagent kits and MiSeq Sequencers, with a minimum target of 3.5 million reads per DNA sample (as per manufacturer's recommendations).

Secondary Analysis Methods: Automated analysis of DNA FASTQ files used Archer Analysis Unlimited Platform v7.1 to create variant output files. After reformatting, variant data was uploaded to Pierian's Clinical Genomic Workspace software for manual classification of findings by Pierian's in-house bioinformatics team.

Variant Calling: Variants are reported according to HGVS nomenclature (www.hgvs.org/mutnomen) and classified as per 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 known polymorphisms.

Additional Notes:

- Copy Number Variation (CNV) was assessed using a group of CNV-normal control samples as a baseline.
- Variants located outside of targeted regions are not detected.
- 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 no representation or warranty of any kind, expressed or implied, regarding the accuracy of the information provided by or contained in such manuscripts, references, and other sources of information. If any of the information provided by or contained in such manuscripts, references, and other sources is later determined to be inaccurate, the accuracy and quality of the Report may be adversely impacted. **Warde Medical Laboratory** is not obligated to notify you of any impact that future scientific or medical research findings may have on the Report.

The Report must always be interpreted and considered within the clinical context, and a physician should always consider the Report along with all other pertinent information and data that a physician would prudently consider 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 a next generation sequencing assay which does not distinguish between somatic and germline variants. If a germline variant is in

question, further testing may be recommended. As such, the relevance of the Report should be interpreted in the context of a patient's clinical manifestations. The Report provided by **Warde Medical Laboratory** is provided on an "AS IS" basis.

Medical knowledge annotation is constantly updated and reflects the current knowledge at the time.

The test performance characteristics were determined by the **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.

REFERENCES

PMID **26322273**: (Palmieri G, *et al.*; Multiple Molecular Pathways in Melanomagenesis: Characterization of Therapeutic Targets.; Front Oncol; 2015;5:183)

PATIENT AND ORDER DETAILS

PATIENT	PHYSICIAN	SPECIMEN	CASE
DATE OF BIRTH	FACILITY	SPECIMEN TYPE Blood specimen	DATE REPORTED
SEX	ORDERING PHYSICIAN	EXT. SPECIMEN ID	
		DATE COLLECTED	
		DATE RECEIVED	

Report electronically reviewed and signed out by

Matthew Sekedat

Date Reported: 02/12/2024