| Patient ID / MRN | Patient Name | | Birth Date | Gender | Age |
|---|---|---|---|---|---|
| | LAST, FIRST | | DD MM YYYY | | |
| Accession Number | Account Name | | Ordering Physician | | |
| | Clinic/Hospital | | LAST, FIRST | | |
| Sample Type | Collected | Received | Reported | | |
| **Text** | DD MM YYYY | DD MM YYYY | DD MM YYYY | | |
| Test | | Test Indication | | | |
| 17243208 - TP53 Full Gene Analysis, NGS, Varies | | Text | | | |

## TP53 Full Gene Analysis, NGS, Varies

### ONCOGENIC OR LIKELY ONCOGENIC (TIER I/II) VARIANTS DETECTED

**TP53: Chr17(GRCh38):g.7675088; NM_000546.6(TP53):c.524G>A; p.Arg175His (30%)**

See below for the Variants of Unknown Clinical Significance and Additional Notes.

### INTERPRETATION

**TP53: Chr17(GRCh38):g.7675088; NM_000546.6(TP53):c.524G>A; p.Arg175His**

**Normal gene/protein function**: The gene TP53 encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism.

**Mutation effect**: The variant c.524G>A; p.Arg175His in TP53 gene is located in the protein's DNA binding domain. In vitro and in vivo studies have demonstrated that this mutation is inactivating and oncogenic, as measured by the reduced ability to induce apoptosis and form colonies, as well as increased cell migration and faster progression of hematopoietic malignancy of the mutant compared to TP53 wildtype or deletion (PMID: 15781620, 25584008, 31068365). Structural studies have also shown that this mutant is defective in protein folding and DNA binding (PMID: 10713666, 21445056). This variant has been reported in several cancer types (https://cancer.sanger.ac.uk/cosmic/mutation/overview?id=103960310, last accessed: 22/11/2024), including Acute Myeloid Leukemia (PMID: 25412851, 31068365, 35380239, 34132463, 23349305).

**Disease associations**: TP53 mutations are universal across cancer types. The loss of a tumor suppressor is most often through truncating, loss of function mutations. In TP53 however, many of the observed mutations in cancer are found to be single nucleotide missense variants. These variants are broadly distributed throughout the gene, but with the majority localizing in the DNA binding domain. Most mutations in the DNA binding domain occur in amino acid positions 175, 245, 248, 273, and 282 (PMID: 20182602). TP53 is also mutated in germline. Germline TP53 mutations are the hallmark of Li-Fraumeni syndrome. The variant p.Arg175His correlated with worse overall survival in cancer patients including hematologic cancer (particularly AML) than wild type TP53, however, it is less detrimental than the p.Arg248Trp variant (PMID: 16489069, 23349305, 29148089, 27288520).

**Therapeutic implications**: At present, there are no approved therapies targeting TP53 mutations, despite their high prevalence in cancer. Inhibition of components of the DNA damage checkpoints have been reported to enhance the activity of DNA-damaging agents in preclinical cancer studies with deficiency of TP53 function (PMID: 21087899, 20107315, 21799033). Additional studies are needed to assess the therapeutic significance of TP53 mutations (including the variant detected in this individual) in cancer.

### VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE

None Detected

| Patient ID / MRN | Patient Name | | Birth Date | Gender | Age |
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The variant(s) listed in this section have insufficient evidence of oncogenicity or clinical significance. They are listed here for future reference in the event they become clinically significant in the light of new scientific evidence.

## METHODOLOGY

The total genomic DNA was extracted from the provided sample using a Bead-based EZ1 DSP DNA Blood Kit (Qiagen, Hilden, Germany). After DNA quality and quantity were assessed using the Denovix DS-11 Spectrophotometer/Fluorometer system, the DNA was randomly fragmented, and ligated sequencing libraries were prepared using the Agilent Magnis NGS Prep system. Regions of interest were targeted by the hybridization-based target capture method using SureSelect CD Glasgow Cancer Haem Panel Kit (Agilent Technologies, CA, USA). Captured DNA was sequenced to an average depth of ≥500X on the Illumina NextSeq 2000 using 2x150 bp paired-end reads (Illumina, San Diego, CA, USA). Primary data analysis converting images into base calls and associated quality scores, and secondary analysis aligning the sequencing reads against the reference human genome (GRCh38/hg38) and variant calling were carried out by using Illumina's proprietary software (Dragen 3.10.12). Quality control analysis of the sequencing data was performed by QualiMap v.2.2.2-dev (Max Planck Institute for Infection Biology, Germany) and VarSeq 2.6.2 (Golden Helix, MT, USA). Copy number variation (CNV) calling was performed on Dragen-CNV (3.10.12) and VarSeq 2.6.2. Variant annotation, filtering, and interpretation were performed on VarSeq 2.6.2.

Variants are annotated following the HGVS (Human Genome Variation Society) nomenclature system. Classification of variants was performed based on AMP/CAP/ASCO and ClinGen guidelines (Li et al, 2017, PMID: 27993330; Horak et al, 2022, PMID: 35101336). AMP/CAP/ASCO guideline classifies the clinical significance of variants into four tiers, namely, Tier I: Variant of Strong Clinical Significance, Tier II: Variant of Potential Clinical Significance, Tier III: Variant of Unknown Clinical Significance, and Tier IV: Benign or Likely Benign Variants. Meanwhile, ClinGen guideline classifies the oncogenicity of the variants into five categories, namely Oncogenic, Likely Oncogenic, Variant of Uncertain Significance, Likely Benign, and Benign. Likely Benign and Benign variants are not reported.

## PERFORMANCE CHARACTERISTICS OF NGS PANEL

Single base substitution: accuracy >99%; reproducibility 100% (intra- and inter-assay); analytical sensitivity: 5% variant allele fraction with a minimum coverage of 250X (2% to 4.99% variant allele fraction with a minimum coverage of 500X will also be reported). Small insertion/deletion events (up to 52 bp): accuracy >99%; reproducibility 100% (intra- and inter-assay); analytical sensitivity: 5% variant allele fraction with a minimum coverage of 250X (2% to 4.99% variant allele fraction with a minimum coverage of 500X will also be reported). Larger single gene insertion/deletion events with size ≥52 bp including (meeting the minimum coverage of 500X and variant allele fraction ≥5%) including loss of TP53 gene and TP53 gene amplification (>4 copies) will also be reported.

## COVERAGE METRICS

Average coverage for TP53 gene: 1350X
Minimum coverage for TP53 gene: 540X

## ADDITIONAL NOTES

None

## CLINICAL TRIALS

| Patient ID / MRN | Patient Name | | Birth Date | Gender | Age |
|---|---|---|---|---|---|
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Information regarding possible clinical trials for this patient can be found at the following sites:

1. ClinicalTrial.gov: https://clinicaltrials.gov/
2. National Cancer Institute: https://www.cancer.gov/research/participate/clinical-trials-search
3. The Leukemia and Lymphoma Society's Clinical Trial Support Center: https://www.hematology.org/education/clinicians/clinical-trial-support-center

## LIMITATIONS

This test does not detect gene fusions, balanced translocations, complex inversions, intronic variants deeper than ±10 base pairs from the exon-intron boundary and copy-neutral loss of heterozygosity (CN-LOH) unless otherwise indicated. Additionally, this test may not reliably detect the following: insertion/deletions larger than 52bp and single exon deletions or duplications.

The depth of sequencing coverage may be variable for some target regions, and they will be noted if they are below the minimum acceptable criteria (minimum reads <50). Low tumor cell percentage in the sample may affect the true variant allele fraction (VAF) and/or sensitivity. This assay does not distinguish between somatic and germline mutations, particularly those with variant allele fractions near 50% or 100%. Similarly, prior treatment for hematological malignancy can affect the results obtained in this assay.

## DISCLAIMER

Because this is a qualitative test, the VAFs provided are for information purposes only and do not indicate a measure of analytical sensitivity for the given genes. The presence or absence of a TP53 mutation may not be predictive of prognosis or response to therapy in all patients. If a detected mutation is suspected to be a germline mutation associated with hereditary diseases, and there is also strong clinical suspicion or a family history of hereditary cancer, additional genetic testing and counseling are recommended.

Reliable results re dependent on adequate specimen collection and processing. This test has been validated on blood, cytology slides and formalin-fixed, paraffin-embedded tissues; other types of fixatives are discouraged. Improper treatment of tissues such as decalcification, may cause PCR failure.

The results of this test must always be interpreted within the context of clinical findings and other relevant data. This test is not intended to detect minimal residual disease. This test was developed, and its performance characteristics were determined by the Molecular Diagnostics & Genomics Laboratory of NRL. This test is used for clinical purposes and should not be regarded as investigational or for research.

## REVIEWED BY

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