| | | | |
|---|---|---|---|
| Patient Name: | | CRM ID: | |
| Age: | | Sample Type: | |
| Sex: | | Sample Collection Date: | |
| Referring Clinician: | | Sample Receiving Date: | |
| Test Requested: | Comprehensive Leukemia Gene Panel by NGS (DNA+RNA) | Reporting Date: | |

# COMPREHENSIVE LEUKEMIA GENE PANEL BY NGS

## CLINICAL INDICATION

Suspected case of acute leukaemia(?ALL).

## RESULT SUMMARY

**POSITIVE**

**(A pathogenic variant detected in ETV6 gene related to clinical phenotype)**

## KEY FINDINGS

| Genes & Transcript | Exons | Depth & VAF | Variant | ASCO/AMP Class with relevance* |
|---|---|---|---|---|
| *ETV6* (NM_001987.5) | Exon 7 | VAF: 18.84% | c.1170_1171insGATACTACC p.Thr390_Tyr391insAspThrThr | IIC |

*Genetic test results are reported based on the recommendations of AMP-ASCO-CAP guidelines.

## Clinical correlation & possible interpretation

Potential relevance: ETV6 mutations are seen in ~2% cases of B-ALL. (PMID: 24997145)

## RECOMMENDATION

1. Please correlate with clinical features, CBC, bone marrow findings, immunophenotyping and cytogenetic for final conclusion.
2. Genetic counseling for accurate interpretation of test results is recommended.
3. For questions about this report, or for assistance in locating nearby genetic counseling services, please contact the Laboratory: geneticcounselors@redcliffelabs.com, ccsupport@redcliffelabs.com.

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Approved by
Dr. Himani Pandey
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## METHODOLOGY & VARIANT ANALYSIS

***Next Generation Sequencing:*** Genomic DNA from the submitted specimen was enriched for the complete coding regions and splice site junctions of genes listed below using a custom bait-capture system. Paired End Sequencing was performed with 2x100/2x150 chemistry, on NGS platform. Reads were assembled and were aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. Data was filtered and analyzed to identify variants of interest and interpreted in the context of a single most damaging, clinically relevant transcript for the purpose of the report, indicated as a part of variant details. Enrichment and analysis focus on the coding sequence of the indicated transcripts, and other specific genomic regions demonstrated to be causative of disease at the time of assay design. Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS).

**Tools and Databases employed for analysis:** Clinvar, OMIM, HGMD, UCSC genome browser, Uniprot, Ensembl, dbSNP, gnomAD, ExAC, Pubmed, Dgap, icgc, Kaviar, various bioinformatics analysis, predictive tools and disease specific databases used as available and appropriate. Such tools/databases would be mentioned wherever used. Please note that RNA sequencing QC metrices were suboptimal (assay repeated), hence a rare possibility of false negative fusion result cannot be excluded. Analysis has been performed on the genes mentioned in attached table.

## TEST LIMITATIONS

- ✓ It should be noted that this test is limited to a limited number of genes and does not include all intronic and non- coding regions.
- ✓ This report only includes variants that meets a level of evidence threshold for cause or contribute to disease. Test results are interpreted in the context of clinical & pathological findings and laboratory data.
- ✓ The accuracy and completeness may vary due to variable information available in different databases. Synonymous mutations were not considered while preparing this report.
- ✓ Variants of unknown significance may be detected and may not be reported subject to analysis by various methodologies. The variants have not been confirmed using Sanger sequencing and/or alternate technologies.
- ✓ Variant with minimum 250x coverage or more as 500x are considered for clinical correlation with the disease. Only mutations having VAF >5% will be reported. PCR primer binding site polymorphisms or mutations might lead to allele dropout & cause false negative results.
- ✓ Mutations in under-performing amplicon (<100x coverage) may be missed. Fusions having deep intronic breakpoints might be missed. Indel exceeding 50 bp size may not be detected.
- ✓ FLT3-ITD has been screened using a fragment length analysis for only those cases which are referred with a suspected diagnosis of AML at the time of sending the sample.
- ✓ The sensitivity of this assay to detect small deletions/duplication is upto certain number of bases only. CNVs if detected with this assay have to be confirmed by alternate method such as MLPA & Micro-array.
- ✓ Variations with high minor allele frequencies which are benign/likely benign will be given upon request if required. Certain genes may not be covered completely, and few mutations could be missed.
- ✓ Due to inherent technology limitations, coverage is not uniform across all regions. Hence, pathogenic variants present in areas of insufficient coverage as well as those variants which currently do not correlate with the provided phenotype may not be analyzed/ reported. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity.
- ✓ This assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements. Incidental or secondary findings (if any) that meet the ACMG/AMP-ASCO-CAP guidelines can be given upon request.

- ✓ Genes with pseudo-genes, para-log genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions. Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS).
- ✓ All laboratory tests are associated with an error rate of ~1%. These could be due to sample mismatch, inappropriate labeling, processing or technological limitations. Please correlate with clinical features and other investigations for final conclusion and send a repeat sample for analysis if necessary.
- ✓ The transcript used for clinical reporting generally represents the canonical transcript, which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.

**DISCLAIMER**

- ❖ Test has been performed assuming that the sample received belongs to the above-named individual(s) and that any stated relationships between individuals are accepted as true. It is also assumed that consent for the same was provided after pre- test counseling at the point of collection/referral.
- ❖ The results should be interpreted in the context of the patient's medical evaluation, family history and racial/ethnic background. Please note that variant classification and/or interpretation may change over time if more information available. Re-interpretation of multi gene next generation sequencing data is recommended on an annual basis and may be requested by a medical provider.
- ❖ More evidence for disease association of genes and causal pathogenic variants are discovered every year, and it is recommended that genetic variants are re-interpreted with updated software and annotations periodically.
- ❖ Rare polymorphisms may lead to false negative or positive results. False negative results may be due to sampling error/errors in sample handling as well as clonal density below the limit of detection. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Identification of a mutation in one or more of these genes does not guarantee activity of the drug in a given indication due to the presence of contraindicated mutation in the gene not covered by the panel.
- ❖ The information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician.
- ❖ Patient care and treatment decisions should only be made by the physician after taking into account all relevant information available including but not limited to the patient's condition, family history, findings upon examination, results of other diagnostic tests, and the current standards of care.
- ❖ This report should only be used as an aid and the physician should employ sound clinical judgment in arriving at any decision for patient care or treatment.
- ❖ By providing drug information for the reported diagnosis, Redcliffe Lab Pvt. Ltd. is not guaranteeing that any drug or clinical trial is necessarily appropriate for this patient.
- ❖ Healthcare providers should evaluate and interpret the information provided in this report, along with all other available clinical information about this patient, to determine the best treatment decisions in their own independent medical judgment. Patient management decisions should not be based on a single test, including this one, nor solely on the information contained in this report.

**VARIANT REPORTING CLASSIFICATION BASED ON AMP-ASCO-CAP RECOMMENDATIONS**

| | |
|---|---|
| **Variants** | A change in a gene. This could be disease causing (pathogenic) or not disease causing(benign). |
| **Tier I** | ***Variants with Strong Clinical Significance (Level A and B Evidence)***<br>**Level A**, biomarkers that predict response or resistance to US FDA-approved therapies for a specific type of tumor or have been included in professional guidelines as therapeutic, diagnostic, and/or prognostic biomarkers for specific types of tumors;<br>**Level B**, biomarkers that predict response or resistance to a therapy based on well- powered studies with consensus from experts in the field or have diagnostic and/or prognostic significance of certain<br>diseases based on well-powered studies with expert consensus. |
| **Tier II** | ***Variants with Potential Clinical Significance (Level C and D Evidence)***<br>**Level C**, biomarkers that predict response or resistance to therapies approved by FDA or professional societies for a different tumor type (i.e., off-label use of a drug), serve as inclusion criteria for clinical trials, or have diagnostic and/or prognostic significance based on the results of multiple small studies. **Level D**, biomarkers that show plausible therapeutic significance based on preclinical studies or may assist disease diagnosis and/or prognosis themselves or along with other biomarkers based on small studies or multiple case reports with no consensus. |
| **Tier III** | ***Variants of Unknown Significance***<br>Not observed at a significant allele frequency in the general or specific sub population or pan cancer or tumor specific variant databases. No convincing published evidence of Cancer Association |
| **Tier IV** | ***Benign or Likely Benign*** |

**APPENDIX: LIST OF INCLUDED GENES**

| Target genes | | | Targeted Fusion genes | | | |
|---|---|---|---|---|---|---|
| ABL1 | JAK2 | PTPN11 | ABL1 | MYH11 | IL3 | |
| ASXL1 | KIT | RARA | JAK2 | TP53 | RUNX1 | |
| BCOR | KMT2A | RB1 | SETBP1 | ETV6 | IL3 | |
| BRAF | KRAS | RBM15 | BCOR | NPM1 | | |
| CALR | MECOM | RUNX1 | KIT | U2AF1 | | |
| CBL | MLLT10 | SETBP1 | SF3B1 | EZH2 | | |
| CEBPA | MLLT3 | SF3B1 | BRAF | NUP214 | | |
| CSF3R | MPL | SH2B3 | KMT2A | WT1 | | |
| DNMT3A | MYD88 | SRSF2 | SRSF2 | FGFR1 | | |
| ETV6 | MYH11 | STAG2 | CALR | NUP98 | | |
| EZH2 | NF1 | TCF3 | MECOM | ZRSR2 | | |
| FGFR1 | NPM1 | TET2 | STAG2 | FLT3 | | |
| FLT3 | NRAS | TP53 | CBL | PDGFRA | | |
| GATA2 | NUP214 | U2AF1 | MLLT10 | GATA2 | | |
| HRAS | NUP98 | WT1 | TCF3 | PDGFRB | | |
| IDH1 | PDGFRA | ZRSR2 | CSF3R | IDH1 | | |
| IDH2 | PDGFRB | | MLLT3 | RARA | | |
| IKZF1 | PHF6 | | TET2 | IDH2 | | |
| IL3 | PRPF8 | | DNMT3A | RBM15 | | |

## REFERENCES

1. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID: 24071849
2. Cerami et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012 May;2(5):401-4. PMID: 22588877
3. 1000 Genomes Project Consortium et al., A global reference for human genetic variation. Nature, 526(7571): 68-74, 2015.
4. Lek M. et al., Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616):285-91, 2016.
5. McLaren, W., et al., Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics, 2010. 26(16): p. 2069-70.
6. Schwarz JM, Cooper DN, Schuelke M and Seelow D. Mutationtaster2: mutation prediction for the deep- sequencing age. NatureMethods.2014; 11: 361-62.
7. Li et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23.
8. Landrum et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res. 2018 Jan 4;46(D1):D1062-D1067. PMID: 29165669

**.....End of Report .....**

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# Terms and Conditions of Reporting

1. The presented findings in the Reports are intended solely for informational and interpretational purposes by the referring physician or other qualified medical professionals possessing a comprehensive understanding of reporting units, reference ranges, and technological limitations. The laboratory shall not be held liable for any interpretation or misinterpretation of the results, nor for any consequential or incidental damages arising from such interpretation.

2. It is to be presumed that the tests performed pertain to the specimen/sample attributed to the Customer's name or identification. It is presumed that the verification particulars have been cleared out by the customer or his/her representation at the point of generation of said specimen / sample. It is hereby clarified that the reports furnished are restricted solely to the given specimen only.

3. It is to be noted that variations in results may occur between different laboratories and over time, even for the same parameter for the same Customer. The assays are performed and conducted in accordance with standard procedures, and the reported outcomes are contingent on the specific individual assay methods and equipment(s) used, as well as the quality of the received specimen.

4. This report shall not be deemed valid or admissible for any medico-legal purposes.

5. The Customers assume full responsibility for apprising the Company of any factors that may impact the test finding. These factors, among others, includes dietary intake, alcohol, or medication / drug(s) consumption, or fasting. This list of factors is only representative and not exhaustive.