

Hematologic Neoplasm Next Generation Sequencing Panel

Background

Recurrent mutations are found in numerous hematologic neoplasms including myelodysplastic syndromes, myeloproliferative neoplasms, acute myeloid leukemia, acute lymphoblastic leukemia, and selected mature lymphoid leukemias.¹⁻⁴ The identification of such mutations provides pathologists and clinicians with useful data that may assist in the diagnosis, classification, prognostic evaluation, and therapeutic management of these malignancies. Mutational data in these disorders has been incorporated into the current diagnostic criteria of the World Health Organization Classification of Hematopoietic and Lymphoid Tissues, and into practice guidelines from the National Comprehensive Cancer Network.^{5,6}

Cleveland Clinic Laboratories offers a next generation sequencing panel that analyzes the clinically relevant regions of 62 genes known to be mutated in hematologic neoplasms. This test, which may be performed on peripheral blood or bone marrow aspirate, identifies single nucleotide variants, insertions and deletions in the targeted genes. Whole genome copy number analysis may also be obtained by concurrently ordering Cancer Chromosome Microarray + SNP testing.

Smaller subpanels are also available for focused disease testing, if desired. The Myeloid Neoplasm Next Generation Sequencing Panel examines 50 genes mutated in myelodysplastic syndromes, myeloproliferative neoplasms, and acute myeloid leukemia. The Myeloid Neoplasm panel includes all 34 genes recommended by the Association for Molecular Pathology for analysis of chronic myeloid neoplasms.⁷ The Acute Lymphoblastic Leukemia panel includes 25 genes recurrently mutated in lymphoblastic leukemias, and the Chronic Lymphoproliferative Disorders panel targets 7 genes mutated in mature lymphoid leukemias including chronic lymphocytic leukemia, lymphoplasmacytic leukemia, hairy cell leukemia and large granular lymphocyte leukemias. Details of the regions covered in all panels are available at clevelandcliniclabs.com.

Clinical Indications

This assay is intended for patients with known or suspected hematologic neoplasms including myelodysplastic syndromes, myeloproliferative neoplasms, acute myeloid leukemia, acute lymphoblastic leukemia, and selected mature lymphoid leukemias.

Interpretation

All variants are classified using Association for Molecular Pathology guidelines for interpretation of somatic variants in cancer.⁸ Detailed interpretations are provided for each variant, and an overall interpretation of the entire mutational profile summarizes the case findings. Reported variants include those of strong or potential clinical significance as well as variants of unclear clinical significance. Known benign polymorphisms are not reported.

Methodology

Nucleic acid extracted from the specimen is subjected to nested multiplex PCR-based target enrichment. Coding and non-coding regions of targeted genes are amplified and sequenced on an Illumina instrument (San Diego, CA) with paired end, 150x2 cycle reads. A customized bioinformatic analytical pipeline is used to map reads to the reference human genome (Genomic Build GRCh37/hg19).

Limitations of the assay

This test does not detect structural variants or copy number changes, and does not distinguish between variants that are inherited versus acquired. During internal validation, this test delivered an average of >500X coverage and >98% of targeted regions showed over 100X coverage. The test demonstrated 95.2% sensitivity and 99.9% specificity in identifying single nucleotide variants, small insertions and deletions (indels) (≤ 10 bp) of >5% variant allele fraction (VAF). For the identification of large indels (>10bp) at >5% VAF the test demonstrated 87.5% sensitivity and 99.9% specificity. Due to limitations of next generation sequencing technology, some large insertions may not be detected.

References

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3. Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. *N Engl J Med*. 2015 Sep 17;373(12):1136-52.
- 4/ Nazha A, Zarzour A, Al-Issa K, et al. The complexity of interpreting genomic data in patients with acute myeloid leukemia. *Blood Cancer J*. 2016 Dec 16;6(12):e510.
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6. "NCCN Guidelines for Treatment of Cancer by Site," National Comprehensive Cancer Network, URL: https://www.nccn.org/professionals/physician_gls/default.aspx#site.
7. McClure RF, Ewalt MD, Crow J, et al. Clinical Significance of DNA Variants in Chronic Myeloid Neoplasms: A Report of the Association for Molecular Pathology. *J Mol Diagn*. 2018 Nov;20(6):717-737. doi: 10.1016/j.jmoldx.2018.07.002. Epub 2018 Aug 20.
8. Li MM, Datto M, Duncavage EJ, 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.

Targeted Gene Regions

| <i>Gene</i> | <i>Transcript</i> | <i>Exon</i> |
|---------------|-------------------|-----------------|
| <i>ABL1</i> | NM_005157.5 | 4-6 |
| <i>ASXL1</i> | NM_15338.5 | 10-13 |
| <i>BCOR</i> | NM_17745.5 | 2-15 |
| <i>BCORL1</i> | NM_021946.4 | 1-12 |
| <i>BRAF</i> | NM_004333.4 | 15 |
| <i>CALR</i> | NM_004343.3 | 9 |
| <i>CBL</i> | NM_005188.3 | 8-9 |
| <i>CDKN2A</i> | NM_000077.4 | 1-2 |
| <i>CDKN2A</i> | NM_058195.3 | 1 |
| <i>CEBPA</i> | NM_004364.4 | 1 |
| <i>CSF3R</i> | NM_000760.3 | 14-17 |
| <i>CUX1</i> | NM_001202543.1 | 15-24 |
| <i>CUX1</i> | NM_001913.4 | 1-23 |
| <i>DDX41</i> | NM_016222.3 | 1-17 |
| <i>DNMT3A</i> | NM_022552.4 | 2-23 |
| <i>EED</i> | NM_003797.4 | 1-12 |
| <i>ETNK1</i> | NM_018638.4 | 3 |
| <i>ETV6</i> | NM_001987.4 | 1-8 |
| <i>EZH2</i> | NM_004456.4 | 2-20 |
| <i>FBXW7</i> | NM_018315.4 | 7-11 |
| <i>FLT3</i> | NM_004119.2 | 14-17, 19-20 |
| <i>GATA1</i> | NM_002049.3 | 2, 4 |
| <i>GATA2</i> | NM_032638.4 | 2-6 |
| <i>GNAS</i> | NM_000516.5 | 8-11 |
| <i>IDH1</i> | NM_005896.3 | 4 |
| <i>IDH2</i> | NM_002168.3 | 4 |
| <i>IKZF1</i> | NM_006060.5 | 2-3, 5-7 |
| <i>JAK2</i> | NM_004972.3 | 12-16 |
| <i>JAK3</i> | NM_000215.3 | 11-18 |
| <i>KDM6A</i> | NM_021140.3 | 1-29 |
| <i>KIT</i> | NM_000222.2 | 2, 8-11, 13, 17 |
| <i>KMT2A</i> | NM_005933.3 | 1-36 |
| <i>KRAS</i> | NM_004985.4 | 2-4 |

| <i>Gene</i> | <i>Transcript</i> | <i>Exon</i> |
|-------------------------|-------------------|-------------|
| <i>LUC7L2 (C7orf55)</i> | NM_001244585.1 | 2-11 |
| <i>MPL</i> | NM_005373.2 | 10-11 |
| <i>MYD88</i> | NM_002468.4 | 5 |
| <i>NF1</i> | NM_000267.3 | 1-57 |
| <i>NF1</i> | NM_001042492.2 | 31 |
| <i>NOTCH1</i> | NM_17617.4 | 26, 27, 34 |
| <i>NPM1</i> | NM_002520.6 | 8-11 |
| <i>NRAS</i> | NM_002524.4 | 2-4 |
| <i>PHF6</i> | NM_001015877.1 | 2-10 |
| <i>PIGA</i> | NM_002641.3 | 2-6 |
| <i>PPM1D</i> | NM_003620.3 | 1-6 |
| <i>PRPF8</i> | NM_006445.3 | 2-43 |
| <i>PTEN</i> | NM_000314.6 | 1-9 |
| <i>PTPN11</i> | NM_002834.3 | 3-4, 12-13 |
| <i>RAD21</i> | NM_006265.2 | 2-14 |
| <i>RIT1</i> | NM_006912.5 | 5 |
| <i>RUNX1</i> | NM_001754.4 | 2-9 |
| <i>RUNX1</i> | NM_001122607.1 | 5 |
| <i>SETBP1</i> | NM_015559.2 | 4* |
| <i>SF3B1</i> | NM_012433.3 | 13-16 |
| <i>SH2B3</i> | NM_005475.2 | 2 |
| <i>SMC1A</i> | NM_006306.3 | 1-25 |
| <i>SMC3</i> | NM_005445.3 | 1-29 |
| <i>SRSF2</i> | NM_003016.4 | 1-2 |
| <i>STAG2</i> | NM_00104279.2 | 3-35 |
| <i>STAT3</i> | NM_003150.3 | 20-21 |
| <i>STAT5B</i> | NM_012448.3 | 16-18 |
| <i>SUZ12</i> | NM_015355.3 | 1-16 |
| <i>TET2</i> | NM_001127208.2 | 3-11 |
| <i>TP53</i> | NM_000546.5 | 2-11 |
| <i>U2AF1</i> | NM_006758.2 | 2, 6 |
| <i>WT1</i> | NM_000378.4 | 1-9 |
| <i>ZRSR2</i> | NM_005089.3 | 1-11 |

* Exon is only partially analyzed from genomic coordinates chr18:42531679-42532175.

Test Overview

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|------------------------------|--|
| Test Name | Hematologic Neoplasm Next Generation Sequencing Panel |
| Ordering Mnemonic | HNPNGS (blood), HNMNGS (bone marrow) Subpanels available: Myeloid – 50 genes ALL – 25 genes Chronic LPD – 7 genes MPN – 3 genes |
| Methodology | Next-generation DNA sequencing |
| Specimen Requirements | 4 mL peripheral blood, EDTA (lavender), or 2 mL aspirate bone marrow, EDTA (lavender) |
| Stability | Ambient: 48 hours Refrigerated: 7 days Frozen: Unacceptable |
| Days Performed | 2 days per week |
| Days Reported | 10 days |
| CPT Code | 81455 |

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