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 63 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 available for focused disease testing:

- Subpanel: **Myeloid Neoplasm Next Generation Sequencing Panel – 50 genes**
  Examines 50 genes mutated in myelodysplastic syndromes, myeloproliferative neoplasms, and acute myeloid leukemia. This panel includes all 34 genes recommended by the Association for Molecular Pathology for analysis of chronic myeloid neoplasms.[7]
- Subpanel: **Acute Lymphoblastic Leukemia Panel – 26 genes**
  Includes 26 genes recurrently mutated in lymphoblastic leukemias.
- Subpanel: **Chronic Lymphoproliferative Disorders Panel – 7 genes**
  Targets seven genes mutated in mature lymphoid leukemias, including chronic lymphocytic leukemia, lymphoplasmacytic leukemia, hairy cell leukemia, and large granular lymphocyte leukemias.
- Subpanel: **Myeloproliferative Neoplasms Panel – 3 genes**
  Detects mutations associated with myeloproliferative neoplasms.

Details of the regions covered in all panels are listed in the Test Directory on 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) (≤10bp) 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

1. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013 Nov 21; 122(22):3616-27.
2. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med*. 2011 Jun 30;364(26):2496-506.
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.
5. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016 May 19;127(20):2391-405.
6. "NCCN Guidelines for Treatment of Cancer by Site," National Comprehensive Cancer Nerwork, 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

Gene	Transcript	Exon
ABL1	NM_005157.5	4-6
ASXL1	NM_15338.5	10-13
BCOR	NM_17745.5	2-15
BCORL1	NM_021946.4	1-12
BRAF	NM_004333.4	15
CALR	NM_004343.3	9
CBL	NM_005188.3	8-9
CDKN2A	NM_000077.4	1-2
CDKN2A	NM_058195.3	1
CEBPA	NM_004364.4	1
CSF3R	NM_000760.3	14-17
CUX1	NM_001202543.1	15-24
CUX1	NM_001913.4	1-23
DDX41	NM_016222.3	1-17
DNMT3A	NM_022552.4	2-23
EED	NM_003797.4	1-12
ETNK1	NM_018638.4	3
ETV6	NM_001987.4	1-8
EZH2	NM_004456.4	2-20
FBXW7	NM_018315.4	7-11
FLT3	NM_004119.2	14-17, 19-20
GATA1	NM_002049.3	2, 4
GATA2	NM_032638.4	2-6
GNAS	NM_000516.5	8-11
IDH1	NM_005896.3	4
IDH2	NM_002168.3	4
IKZF1	NM_006060.5	2-3, 5-7
JAK2	NM_004972.3	12-16
JAK3	NM_000215.3	11-18
KDM6A	NM_021140.3	1-29
KIT	NM_000222.2	2, 8-11, 13, 17
KMT2A	NM_005933.3	1-36
KRAS	NM_004985.4	2-4
LUC7L2 (C7orf55)	NM_001244585.1	2-11

Gene	Transcript	Exon
MPL	NM_005373.2	10-11
MYD88	NM_002468.4	5
NF1	NM_000267.3	1-57
NF1	NM_001042492.2	31
NOTCH1	NM_17617.4	26, 27, 34
NPM1	NM_002520.6	8-11
NRAS	NM_002524.4	2-4
PAX5	NM_016734.2	1-10
PHF6	NM_001015877.1	2-10
PIGA	NM_002641.3	2-6
PPM1D	NM_003620.3	1-6
PRPF8	NM_006445.3	2-43
PTEN	NM_000314.6	1-9
PTPN11	NM_002834.3	3-4, 12-13
RAD21	NM_006265.2	2-14
RIT1	NM_006912.5	5
RUNX1	NM_001754.4	2-9
RUNX1	NM_001122607.1	5
SETBP1	NM_015559.2	4*
SF3B1	NM_012433.3	13-16
SH2B3	NM_005475.2	2
SMC1A	NM_006306.3	1-25
SMC3	NM_005445.3	1-29
SRSF2	NM_003016.4	1-2
STAG2	NM_00104279.2	3-35
STAT3	NM_003150.3	20-21
STAT5B	NM_012448.3	16-18
SUZ12	NM_015355.3	1-16
TET2	NM_001127208.2	3-11
TP53	NM_000546.5	2-11
U2AF1	NM_006758.2	2, 6
WT1	NM_000378.4	1-9
ZRSR2	NM_005089.3	1-11

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

Cleveland Clinic

Cleveland Clinic Laboratories

Test Overview

Test Name	Hematologic Neoplasm Next Generation Sequencing Panel
Ordering Mnemonic	HNPNGS (blood), HNMNGS (bone marrow) Subpanels available: **Myeloid Panel – 50 genes** Bone Marrow: MYNGSM Peripheral Blood: MYNGSP **Acute Lymphoblastic Leukemia (ALL) Panel – 26 genes** Bone Marrow: ALLBM Peripheral Blood: ALLPB **Chronic Lymphoproliferative Disorders (LPD) Panel – 7 genes** Bone Marrow: LPMNGS Peripheral Blood: LPPNGS **Myeloproliferative Neoplasms Panel – 3 genes** Bone Marrow: MPNM Peripheral Blood: MPNP
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

Technical Information Contact:

Kristen G. McDonnell, MB(ASCP)^{CM}CG^{CM}
216.314.1008
mcdonnk3@ccf.org

Scientific Information Contact:

David Bosler, MD
216.636.9615
boslerd@ccf.org