Cleveland Clinic

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CC-SIGN Targeted Oncology Panel (TOP) by Next-Generation Sequencing

Overview

Recurrent somatic alterations are found in a variety of solid tumors. Identifying such alterations provides pathologists and clinicians with valuable data that may assist in the diagnosis, classification, prognostic evaluation, and therapeutic management of these malignancies.

The Targeted Oncology Panel (TOP) is a custom 59-gene Next-Generation Sequencing (NGS) panel developed for the identification of single nucleotide variants (SNVs), small insertions and deletions (indels), and copy number gains (CNVs) from DNA specimens, and fusion transcripts and aberrant transcripts from RNA specimens. The workflow allows for the concurrent testing of DNA and RNA to analyze over 1,000 biomarkers.

Besides providing useful information for cancer evaluation and diagnosis, this test can identify pan-cancer therapeutic markers, including:

TOP Biomarker	FDA-Approved Treatment Examples*
ALK fusions	Alectinib, Brigatinib, Ceritinib, Crizotinib for NSCLC
ALK oncogenic mutations	Lorlatinib for NSCLC
BRAF V600E	Vemurafenib, Dabrafenib and others for Melanoma Dabrafenib + Trametinib for all solid tumors
EGFR exon 19 in-frame deletions	Gefitinib, Osimertinib for NSCLC
EGFR exon 20 in-frame insertions	Amivantamab, Mobocertinib for NSCLC
ERBB2 (HER2) amplification	Multiple therapy options for Breast Cancer Tucatinib + Trastuzumab for CRC Trastuzumab options for Esophagogastric Cancer
FGFR2 fusions	Erdafitinib for Bladder Cancer Futibatinib, Pemigatinib for Cholangiocarcinoma
FGFR3 fusions or activating mutations	Erdafitinib for Bladder Cancer
IDH1 R132	Ivosidenib for Cholangiocarcinoma
KIT oncogenic mutations	Imatinib, Regorafenib, Ripretinib, Sunitinib for GIST
KRAS G12C	Adagrasib, Sotorasib for NSCLC
MET exon 14 skipping alterations or amplification	Capmatinib, Tepotinib for NSCLC
NTRK1/2/3 fusions	Entrectinib, Larotrectinib for all solid tumors
PDGFRA exon 18 alterations	Avapritinib for GIST
PIK3CA oncogenic mutations	Alpelisib + Fulvestrant in Breast Cancer
RET fusions	Selpercatinib for all tumors
RET mutations	Pralsetinib, Selpercatinib for Medullary Thyroid Cancer
ROS1 fusions	Crizotinib, Entrectinib for NSCLC

*For illustration purposes only, this table is not comprehensive, nor should it be used as a recommendation for treatment. TOP report does not provide therapy recommendations. Clinical and histopathological correlation is required.

This panel is optimized to evaluate scant specimens, such as cytology specimens and limited biopsies; as such, TOP may be used for advanced tumor profiling and clinical trial evaluation when material is too limited to send for comprehensive sequencing. For example, TOP can detect resistance mutations in *EGFR*, *KRAS*, *NRAS*, and *PDGFRA*, as well as evolving biomarkers in *AKT1*, *BRAF* non-V600, *CDK4*, *ERBB2*, *HRAS*, *MAP2K1/2*, *MTOR*, amongst others.

Clinical Indications

Hotspots, copy number variants, and selected fusions evaluated by this assay can aid in the diagnostic and therapeutic assessment of a variety of tumor types, including non-small cell lung cancer, melanoma, colorectal cancer, prostate cancer, breast cancer, glioblastoma, thyroid cancer, and others. This panel can also provide focused tumor profiling for patients with locally advanced/metastatic disease, who are candidates for anti-cancer therapy, to identify uncommon but targetable alterations.

Interpretation

Variants are classified according to established guidelines, and an interpretation is provided. Reported variants include those of strong or potential clinical significance and variants of unclear clinical significance. Common population variants are not included in the report.

Methodology & Limitations

Extracted nucleic acid from the specimen, both DNA and RNA, were subjected to separate targeted amplification reactions using AmpliSeq custom primers designed by Thermo Fisher Scientific (Thermo Fisher Scientific, Waltham, MA). Hotspots and selected fusions in gene regions listed below were sequenced using Illumina (San Diego, CA) 2x150 paired-end cycle chemistry. A customized bioinformatics analytical platform was used for read alignment (Genome Build GRCh37/hg19), variant identification, and annotation. Single nucleotide variants (SNVs), insertion, deletion (indels), and copy number gain variants are detected by DNA sequencing. Select fusions and aberrant transcripts (EGFR vIII and MET exon 14 skipping transcripts) are detected by RNA sequencing. Variants are classified according to established guidelines. Reported results include variants of strong or potential clinical significance and variants of unclear clinical significance. Benign population polymorphisms are not included in the report.

Based on validation, the DNA testing delivered an average of >500x coverage, and >99% of targeted regions showed over 100x coverage. A minimum coverage depth of 100 reads is required across the entire region of interest; a list of low-coverage areas is included in the report as applicable. The test demonstrated 100% sensitivity and 100% specificity in identifying SNVs, indels, and copy number gains. The lower limit of detection of this assay is approximately 5% variant allele fraction (VAF) for SNV/indels and 6 copies or greater for copy number gains. Variants below these thresholds may be reported at the discretion of the molecular pathology professional staff if the technical quality of the sequencing is sufficient at that location and the call is unequivocal.

Based on validation, the RNA fusion testing averaged >150,000 total reads. The test demonstrated 93% sensitivity and 100% specificity in gene fusion identification compared to NGS sequencing and 69% sensitivity and 100% specificity compared to fluorescence *in situ* hybridization (FISH) of fusion drivers (unknown fusion partner). Overall sensitivity is 78%, and accuracy is 99%. The lower limit of detection is approximately 1% of total sequencing reads.

Sequence changes outside the analyzed alterations hotspots, including intronic and noncoding regions, will not be identified by this test. Insertions and deletions larger than 20 and 40 bp, respectively, may not be identified by this test. Negative results from specimens for which the percentage of tumor cells is 10% or less should be interpreted with caution. Although variant allele fraction is provided as a percentage, this is not a quantitative test. RNA fusions involving alternative partners or breakpoints outside of the targeted regions cannot be detected by this test. This test does not distinguish between somatic and inherited variants. Tumor heterogeneity, tumor burden, specimen degradation or other limitations of the technology may affect the sensitivity and limit of detection, either broadly across the regions of interest or for specific regions, and may lead to false negative results. This test is not intended to be used for circulating tumor evaluation.

TOP Content

Genes & Hotspot Regions Covered for SNV/Indels:

Gene	Transcript	Exon(s)*	Gene	Transcript	Exon(s)*
AKT1	NM_005163	3	*IDH1*	NM_005896	4
ALK	NM_004304	21-25	*IDH2*	NM_002168	4
AR	NM_000044	6, 8	*JAK1*	NM_002227	14 - 16
BRAF	NM_004333	11, 15	*JAK2*	NM_004972	14
CDK4	NM_000075	2	*JAK3*	NM_000215	11-12, 15
CTNNB1	NM_001904	3, 7-8	*KIT*	NM_000222	8-11, 13, 17
DDR2	NM_006182	5	*KRAS*	NM_033360	2-4
EGFR	NM_005228	3 ,7, 12, 15, 18-21	*MAP2K1*	NM_002755	2-3, 6
ERBB2	NM_004448	8, 17-22	*MAP2K2*	NM_030662	2
ERBB3	NM_001982	2-3, 6, 8-9	*MET*	NM_000245	14, 16, 19
ERBB4	NM_005235	18	*MTOR*	NM_004958	30, 39-40, 43, 47, 53
ESR1	NM_000125	8	*NRAS*	NM_002524	2-4
FGFR1	NM_023110	12-14, 16-17	*PDGFRA*	NM_006206	12, 14, 18
FGFR2	NM_000141	7-9, 12, 14	*PIK3CA*	NM_006218	2, 5-6, 8, 10, 14, 19, 21
FGFR3	NM_000142	7, 9, 14, 16	*RAF1*	NM_002880	7, 12
GNA11	NM_002067	4, 5	*RET*	NM_020975	11, 13, 15-16
GNAQ	NM_002072	4,5	*ROS1*	NM_002944	36, 38
GNAS	NM_080425	8	*SMO*	NM_005631	4, 6, 8-9
H3-3A	NM_002107	2	*TERT*	NM_198253	Promoter
H3-3B	NM_005324	2	*TP53*	NM_000546	5, 7, 8
HRAS	NM_005343	2, 3			

*Only hotspots within designated exons are covered, full exons are not sequenced.

Genes reported for copy number gains: *ALK, AR, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, KIT, KRAS, MET, MYC, MYCN, PDGFRA, PIK3CA*

Fusions detected in RNA:

Gene	Detected Fusions
ABL1	*EML1::ABL1*
AKT3	*MAGI3::AKT3*
ALK	*A2M::ALK, ACTG2::ALK, ALK::PTPN3, ATIC::ALK, C2orf44::ALK, CARS::ALK, CLIP4::ALK, CLTC::ALK, DCTN1::ALK, EML4::ALK, GTF2IRD1::ALK, HIP1::ALK, KIF5B::ALK, KLC1::ALK, MEMO1::ALK, NCOA1::ALK, PRKAR1A::ALK, RANBP2::ALK, SEC31L1_SEC31A::ALK, SMEK2::ALK, STRN::ALK, TFG::ALK, TPM1::ALK, TPM3::ALK, TPM4::ALK, TPR::ALK, TRAF1::ALK, VCL::ALK*
AXL	*AXL::MBIP*
BRAF	*AGTRAP::BRAF, AKAP9::BRAF, CDC27::BRAF, FAM131B::BRAF, FCHSD1::BRAF, KIAA1549::BRAF, PAPSS1::BRAF, SLC45A3::BRAF, SND1::BRAF, TAX1BP1::BRAF, TRIM24::BRAF*
EGFR	*EGFR* vIII transcript
ERBB2	*WIPF2::ERBB2*
ERG	*SLC45A3::ERG, TMPRSS2::ERG*
FGFR1	*BAG4::FGFR1, ERLIN2::FGFR1, FGFR1::TACC1*
FGFR2	*FGFR2::AFF3, FGFR2::BICC1, FGFR2::CASP7, FGFR2::CIT, FGFR2::KIAA1967_CCAR2, FGFR2::MGEA5, FGFR2::OFD1, FGFR2::TACC1, SLC45A3::FGFR2*
FGFR3	*FGFR3::AES, FGFR3::BAIAP2L1, FGFR3::ELAVL3, FGFR3::TACC3*
MET	*MET* Exon 14 Skipping, *BAIAP2L1::MET, C8orf34::MET, CAPZA2::MET, OXR1::MET, TFG::MET, TPR::MET, PTPRZ1::MET*
NTRK1	*BCAN::NTRK1, CD74::NTRK1, CEL::NTRK1, IRF2BP2::NTRK1, LMNA::NTRK1, MPRIP::NTRK1, NFASC::NTRK1, NTRK1::DYNC2H1, RNF213::NTRK1, SQSTM1::NTRK1, SSBP2::NTRK1, TFG::NTRK1, TPM3::NTRK1, TPR::NTRK1*
NTRK2	*AFAP1::NTRK2, AGBL4::NTRK2, NACC2::NTRK2, QKI::NTRK2, SQSTM1::NTRK2, TRIM24::NTRK2, VCL::NTRK2*
NTRK3	*BTBD1::NTRK3, COX5A::NTRK3, ETV6::NTRK3*
PDGFRA	*SCAF11::PDGFRA*
PPARG	*PAX8::PPARG*
RAF1	*B4GALT1::RAF1, ESRP1::RAF1*
RELA	*C11orf95::RELA*
RET	*ACBD5::RET, AFAP1::RET, AKAP13::RET, CCDC6::RET, CUX1::RET, ERC1::RET, FKBP15::RET, GOLGA5::RET, HOOK3::RET, KIAA1468::RET, KIF5B::RET, KTN1::RET, NCOA4::RET, PCM1::RET, PRKAR1A::RET, RUFY2::RET, SPECC1L::RET, TBL1XR1::RET, TRIM24::RET, TRIM27::RET, TRIM33::RET*
ROS1	*CCDC6::ROS1, CD74::ROS1, CEP85L::ROS1, CLIP1::ROS1, CLTC::ROS1, ERC1::ROS1, EZR::ROS1, GOPC::ROS1, HLA_A::ROS1, KDELR2::ROS1, KIAA1598::ROS1, LRIG3::ROS1, MSN::ROS1, MYO5A::ROS1, PPFIBP1::ROS1, PWWP2A::ROS1, SDC4::ROS1, SLC34A2::ROS1, TFG::ROS1, TPM3::ROS1, ZCCHC8::ROS1*
TMPRSS2	*TMPRSS2::ERG, TMPRSS2::ETV1, TMPRSS2::ETV4, TMPRSS2::ETV5*

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Test Overview

Test Name	Targeted Oncology Panel by NGS
Order Codes	TOPTO (FFPE Tissue) TOPCY (Cytology Alcohol or Formalin fixed cell block) TOPBM (Bone Marrow Aspirate) TOPPB (Peripheral Blood)
Methodology	Next-Generation Sequencing
Specimen Requirements	**FFPE Tissue** 15 charged, unbaked, and unstained slides sectioned at 7 μm. One pre and one post H&E slide with tumor area circled, and percent tumor indicated. **Cytology** 15 charged, unbaked and unstained slides sectioned at 7 μm. One H&E slide with tumor percent indicated. **Bone Marrow Aspirate** 2 mL, EDTA (lavender) **Peripheral Blood** 4 mL, EDTA (lavender)
Stability	**FFPE Tissue** Ambient: Indefinitely Refrigerated: Indefinitely Frozen: Unacceptable **Cytology Alcohol or Formalin-Fixed Cell Block** Ambient: Indefinitely Refrigerated: Indefinitely Frozen: Unacceptable **Bone Marrow or Peripheral Blood** Ambient: 48 hours Refrigerated: 3 days Frozen: Unacceptable
Days Performed	2–3 times per week
Turnaround Time	8 days
CPT Code	81445

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