TGen Clinical Laboratory Assay Description

ALTseq is a rapid turnaround whole genome sequencing test to support clinical management of Acute Myeloid Leukemia (AML) patients developed by TGen Clinical Lab (TCL). The test can detect single nucleotide variants (SNVs), small insertions and deletions, gene-level and large-scale copy number alterations (CNA) reflecting regions commonly assessed by FISH, and structural variants (SVs) including deletions, inversions, and translocations. FLT3 internal tandem duplication (FLT3-ITD) and KMT2A partial tandem duplication (KMT2A-PTD) events are also detected. The ALTseq clinical report can aid physicians in classifying a patient's AML tumor, determining the prognosis of the disease, and identifying FDA-approved therapies that target specific genetic variants. The ALTseq report focuses on prognostically informative and biologically relevant variants in the context of AML. This test was designed to identify the indicated variant types when the AML blast percentage meets or exceeds 20%. If a specimen's performance within the test is insufficient to properly call CNAs, no CNA information is reported, and this is indicated on the final report. This test is not intended to diagnose AML or related hematological malignancies, nor to monitor disease progression or to identify minimal residual disease.

Genomic DNA is isolated from whole blood or whole bone marrow specimens sourced from acute myeloid leukemia (AML) patients. PCR-free libraries are constructed using Watchmaker Genomics DNA Library kit with fragmentation and sequenced using an Illumina NovaSeq 6000. Sequencing reads are analyzed using a TCL-specific workflow that aligns read pairs to the GRCh38 reference genome and produces variant annotations corresponding to Ensembl 107 MANE transcripts. For genes without a MANE transcript, variant annotation is provided using the canonical transcript from Ensembl. Allele frequencies for SNV and SV along with the inferred copy number can be influenced by the tumor purity, which is not inferred or used to adjust event frequencies. Please note that structural variant allele frequencies are reported as the percentage of total DNA sequenced (in the same manner as SNVs and small indels), not the percentage of cells carrying that variant (as typically reported in FISH reports).

The minimum coverage of each test must meet or exceed 60x. The median coverage for ALTseq tests is 107x. The limit of detection of the assay at 60x for each variant type is as follows: SNV – 8.5% variant allele frequency (VAF), small indel – 13% VAF, CNA – 10% VAF, and SV – 5.0% VAF.

The ALTseq assay has been shown through orthogonal testing to have a precision of 100% for all variant types. The sensitivity for each variant type is as follows: SNVs – 95.5%, small indels – 95.9%, structural variants – 100%, CNAs – 96.4%, and FLT3-ITDs – 95.2%.

Reportable Variants

The following 40 genes are curated in the ALTseq knowledge base for reporting in the ALTseq test: ASXL1, BCOR, BCORL1, BRAF, CALR, CBL, CEBPA, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FLT3, GATA2, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PHF6, PIGA, PPM1D, PTPN11, RAD21, RUNX1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, SUZ12, TET2, TP53, U2AF1, WT1, and ZRSR2. The following structural variants are also evaluated for reporting: BCR::ABL1, CBFB::MYH11, DEK::NUP214, KAT6A::CREBBP, KMT2A rearrangements (109 specific rearrangement partners as well as general KMT2A-rearranged reporting), MECOM rearrangements (GATA2::MECOM as well as general MECOM-rearranged reporting), NUP98 rearrangements (34 specific rearrangement partners as well as general NUP98-rearranged reporting), PML::RARA, RBM15::MRTFA, RUNX1::RUNX1T1, and RUNX1::PRDM16. Please note that structural variant allele frequencies are reported as the percentage of total DNA sequenced (in the same manner as SNVs and small indels), not the percentage of cells carrying that variant (as typically reported in FISH reports). When the specimen is of sufficient quality to call copy number variants, large deletions on chromosomes 5, 7, or 17 are reported as "key genomic findings", gene-level copy number variants involving any reportable gene are reported as a VUS, and a genome-wide CNA plot is provided.

Disclaimers

This test is not designed to diagnose or treat any disease or illness. Samples with a blast percentage lower than 20% may result in false negative results. No therapeutic listed on the report is guaranteed to be effective in the treatment of the disease, and use of these drugs is at the sole discretion of the treating physician. TGen Clinical Laboratory is not liable for medical judgment related to patient care and diagnosis, prognosis, or treatment decisions made in connection with the ALTSeq test results. Genomic results should be considered in the context of the patient's history and risk factors. The test results and information presented in the report are considered current based on the date of the report. TGen Clinical laboratory will not update reports or send notification regarding reclassification of genomic alterations.

This clinical test was developed, and its performance characteristics were evaluated by TGen Clinical Laboratory, and it is not an FDA-approved test. TGen Clinical Laboratory is CLIA-certified and accredited by the College of American Pathologists to perform high-complexity clinical laboratory testing.