Tumor-Agnostic Panel

Although the genetic alterations listed above are historically associated with specific tissues, testing is now increasingly performed regardless of tumor origin. RNA sequencing, as performed routinely at our center and increasingly at many others, has become a key diagnostic tool due to its ability to detect all gene fusions comprehensively.

A comprehensive panel covering all eight biomarkers listed above and generating broader molecular information includes:

TMB + RNA-Seq (all fusions) + HER2 (with optional PD-L1 IHC, if requested).

Ideal Panel Approach

The most comprehensive approach includes:

Tumor-Agnostic Panel + Whole Genome Sequencing (WGS)

- Germline familial cancer panel
- Additional disease screening
- Germline pharmacogenetics

This combined approach not only identifies tumor-specific alterations but also detects inherited genetic variants, drug metabolism profiles, and additional conditions that may influence treatment decisions.

Rationale for Adding Whole Genome Sequencing

- The tumor-agnostic panel is now considered standard practice in many cancers.
- The prevalence of hereditary (familial) cancer is approximately 8–15%.
- The prevalence of rare diseases in the general population that may lead to complications is approximately 10%.
- Individuals differ significantly in drug efficacy and toxicity profiles due to genetic variation.

For these reasons, integrating Whole Genome Sequencing provides a more comprehensive and personalized precision oncology approach.

Major Tumor-Agnostic Tests and Biomarkers

1. MSI-H / dMMR (Microsatellite Instability – DNA Mismatch Repair Deficiency)

Clinically relevant in colorectal, endometrial, gastric, biliary tract, pancreatic, prostate, thyroid, lung, and other solid tumors. Strongly associated with response to immunotherapies (e.g., pembrolizumab).

Testing methods: PCR, IHC, NGS.

2. TMB-H (Tumor Mutational Burden – High Tumor Mutation Load) (≥10 mut/Mb, validated panel)

May serve as a predictive biomarker across multiple cancer types. Important for predicting response to immunotherapy.

Testing method: NGS-based panels.

3. NTRK Gene Fusions (NTRK1, NTRK2, NTRK3)

Observed in various rare solid tumors and pediatric tumors.

Targeted therapies: Larotrectinib, Entrectinib.

Testing methods: NGS, FISH, RT-PCR, IHC.

4. RET Fusions

Detected in thyroid carcinoma, lung adenocarcinoma, and rare tumors.

Targeted therapies: Selpercatinib, Pralsetinib.

5. ALK and ROS1 Fusions

Commonly known in lung adenocarcinoma but also defined as tumor-agnostic alterations in rare tumors.

Targeted therapies: Crizotinib, Entrectinib, Lorlatinib.

6. BRAF V600E Mutation

Seen in melanoma, lung, colorectal, thyroid, brain tumors, and many other cancers.

Targeted therapy: Dabrafenib + Trametinib combination.

7. HER2 (ERBB2) Amplification or Mutation

Detected in breast, gastric, lung, biliary tract, colorectal, and other tumors.

Targeted therapies: Trastuzumab, Trastuzumab deruxtecan.

8**. KRAS G12C Mutation**

Primarily observed in lung and colorectal cancer but may also be found in other tumor types.

Targeted therapies: Sotorasib, Adagrasib.

CANCER GENETIC TESTS

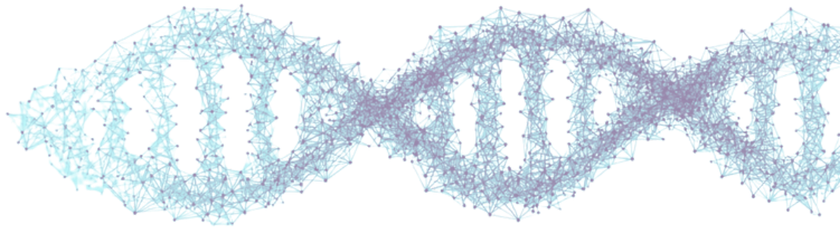
TUMOR-AGNOSTIC APPROACH

TUMOR-AGNOSTIC TESTS SHOULD BE USED IN ALL CANCERS.

A tumor-agnostic test refers to molecular analyses that should be performed independently of tumor tissue type.

Rationale:

- “Rare cancers” constitute approximately 24% of all cancers, and in these patients, the most powerful diagnostic tool is the tumor-agnostic panel.
- Different mutations, as well as variable drug resistance and sensitivity profiles, may be observed in subclonal tumor cell populations.
- Histopathology alone may not always be sufficiently discriminative in guiding treatment selection or determining prognosis.



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What Is the Tumor Mutational Burden (TMB) Test?

The Tumor Mutational Burden (TMB) test performed at our center is based on a panel comprising more than 600 genes. This panel is analyzed using Next-Generation Sequencing (NGS) and includes the detection of SNVs (single nucleotide variants), CNVs (copy number variations), and selected gene fusions. Within this panel, Tumor Mutational Burden (TMB), Microsatellite Instability (MSI), and PD-L1 are also evaluated.

Our reports provide therapeutic guidance based on the detected variants, including drug information and pharmacogenetic insights (drug resistance, sensitivity, and toxicity). In addition to targeted therapy selection, assessments are made regarding chemotherapy sensitivity, resistance, adverse effect risk, and drug metabolism rates.

Tumor Mutational Burden (TMB) testing measures the number of genetic mutations present in cancer cells. A higher number of mutations may make cancer cells more recognizable to the immune system, which is associated with a potentially improved response to immunotherapy.

MSI (Microsatellite Instability) testing is a genetic analysis used to detect alterations in repetitive microsatellite regions of DNA resulting from defects in DNA mismatch repair mechanisms. MSI is most commonly associated with colorectal cancer; however, because it can influence treatment decisions, it is now recommended to be evaluated in all tumor types. Defects in DNA repair mechanisms may trigger genetic alterations that promote cancer cell growth and proliferation.

What Does the TMB Panel Include?

- Microsatellite Instability (MSI)
- Tumor Mutational Burden (TMB)
- BRAF V600E mutation
- Chemotherapy resistance, sensitivity, toxicity, and drug metabolism rate evaluation

Who Should Be Tested?

- All cancer types
- Advanced-stage solid tumors
- Rare or atypical histologies
- Cancer of Unknown Primary (CUP)
- Cases with exhausted standard treatment options

Preferably, testing should be performed using Comprehensive Genomic Profiling (CGP).

Health for **Human,**
Science for **Health**

What Is RNASEQ?

RNA sequencing (RNASEQ) is a technology that enables the sequencing of all RNA molecules expressed within a cell at a given time and allows quantitative comparisons based on defined parameters. Compared to data obtained from DNA sequencing, RNA-based analyses provide insights into the dynamic nature of the cell and ongoing biological processes. RNA sequencing has now become an integral part of both routine clinical practice and research applications.

Key Applications Include:

- Elucidation of disease mechanisms
- Identification of novel drug targets
- Determination of diagnostic and prognostic biomarkers
- Functional interpretation of variants detected at the DNA level
- Identification of alternative transcripts (alternative splicing)
- Detection of fusion/translocation products
- Identification of RNA variants
- Analysis of non-coding RNAs

RNA sequencing has become part of routine diagnostic, therapeutic, and prognostic evaluation particularly in leukemia and lymphoma. In routine practice for leukemia and lymphoma, analyses are most commonly performed using FISH and real-time PCR methods, focusing on specific fusions, deletions, and duplications. In other words, only selected target regions are examined. The parameters evaluated by FISH and real-time PCR are chosen based on their established impact on diagnosis and prognosis.

In contrast, RNA sequencing enables not only the detection of known clinically significant fusions but also the identification of novel immunotherapy candidate genes, as well as genes and variants that may influence prognosis and disease monitoring. All gene fusions, intragenic inversions, duplications, and gene expression levels can be analyzed using this method. Therefore, RNA sequencing has demonstrated highly successful outcomes, especially in cases where a definitive diagnosis cannot be established, where the clinical course is unexpected, where there is resistance to therapy, or where complications arise during treatment.

RNA sequencing can also reveal certain copy number changes, such as deletions and duplications. Since fusion genes may arise not only from translocations and inversions but also from deletions and duplications, it becomes possible—particularly in leukemia—to detect prognostically significant gene deletions and duplications.

Furthermore, RNA sequencing can identify all fusion anomalies and their functional consequences, including alterations frequently observed in leukemias. When performed in combination with exome sequencing, it enables the detection of familial leukemia and cancer predisposition syndromes, and provides the potential to identify most leukemia-associated variants—including polysomies—along with their functional impact.

Why Is It Important?

- In many cancers, kinase fusions (e.g., ALK, ROS1, RET, NTRK, FGFR2/3) directly enable targeted therapy options.
- RNA-based approaches are superior in terms of sensitivity and clinical interpretation in fusion analysis.
- RNASEQ does not require prior assumptions regarding the type of fusion; it provides comprehensive screening.

Who Should Be Tested?

- Advanced-stage solid tumors (particularly cases seeking targeted treatment options)
- Atypical/rare histologies or Cancer of Unknown Primary (CUP)
- Sarcomas
- Cases with suspected pan-TRK/ALK/ROS1 positivity on IHC
- Cases with suspected fusion signals or clues detected in previous DNA panels

What Can Be Detected?

Actionable fusions:

ALK, ROS1, RET, NTRK1/2/3, FGFR2/3, NRG1, specific BRAF fusions, certain MET fusions, etc.

Diagnostic fusions:

EWSR1-FLI1 (Ewing sarcoma),
PAX3/7-FOXO1 (alveolar rhabdomyosarcoma),
KIAA1549-BRAF (pediatric glioma), etc.

Sample Requirements

Preferred: FFPE block or 5–10 tissue sections (4–5 µm), ideally with ≥20% tumor content.
If tissue is insufficient: Cytology cell-block samples may be acceptable.
(Liquid biopsy has limited utility for fusion detection; evaluated on a case-by-case basis.)

What You Will See in the Report

- Fusion name and involved exons (e.g., EML4-ALK (E13;A20))
- Supporting evidence: number of split and/or spanning reads
- Biological relevance: in-frame status and preservation of the 3' kinase domain
- Clinical interpretation

Key Consideration for Clinical Decision-Making

In kinase fusions, if the 3' kinase domain is preserved and the fusion is in-frame, the likelihood of benefiting from targeted therapy is high.