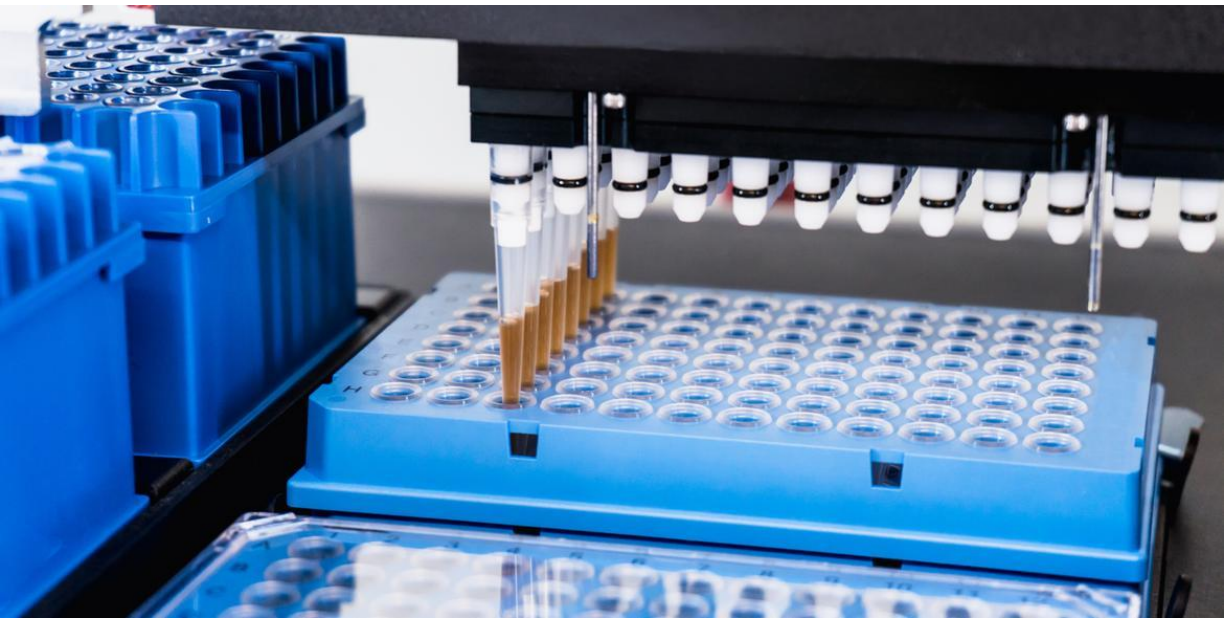
MOLECULAR HEALTH

Molecular pathology / Oncology

Panel diagnostics instead of hotspot? Here are the differences

NGS, WGS, SNV – What do these abbreviations stand for in molecular pathological diagnostics? What are the benefits of NGS-based panel diagnostics in oncology? This overview provides answers regarding methods of molecular tumor diagnostic testing.



Pathways of molecular tumor diagnostics at a glance

This structured classification of methods in tumor diagnostics provides answers to these initial questions:

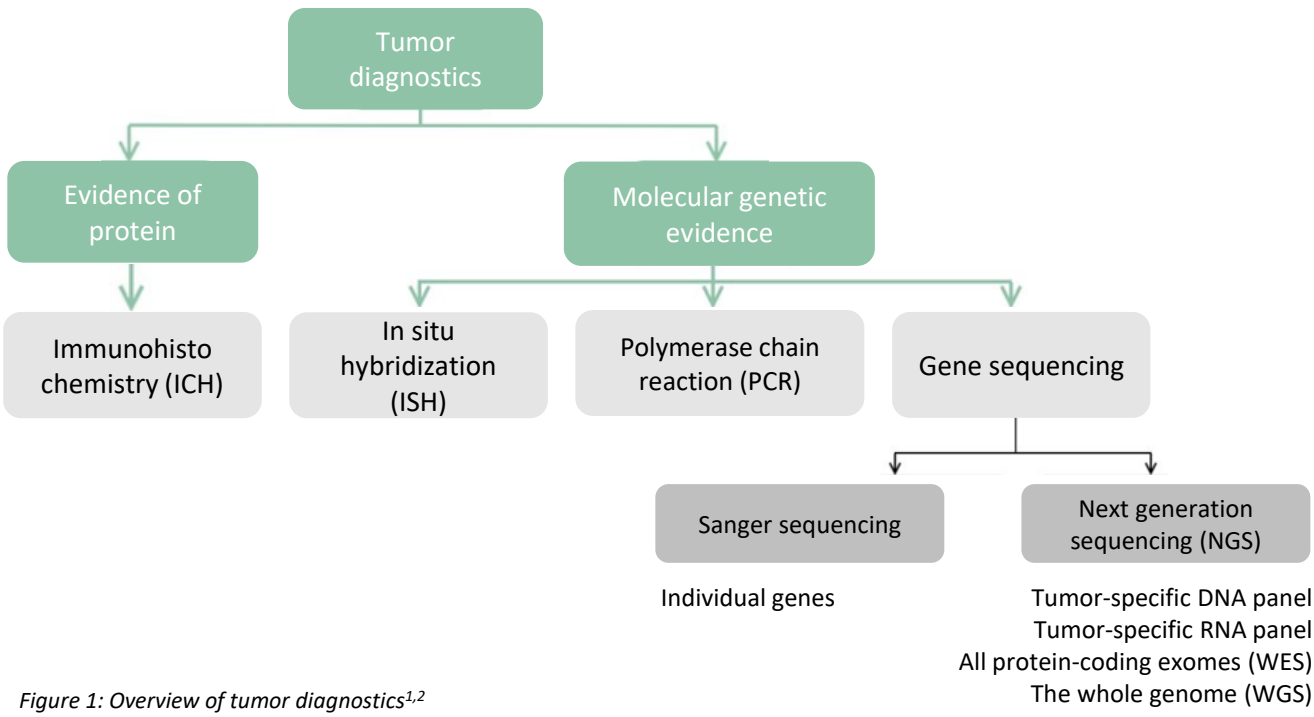


Figure 1: Overview of tumor diagnostics[1,2]

NGS widens the molecular tumor diagnostics spectrum

Next generation sequencing (NGS) includes high-throughput methods which analyze millions of gene sequences in a short period of time and in parallel.[2] Depending on the clinical problem, NGS-based molecular diagnostics enables analysis of

- the DNA or RNA of a specific number of genes (panel diagnostics),[2]
- of more than 20,000 functional genes, i.e. all protein-coding exomes (whole exome sequencing, WES),[2]
- of the entire genome, i.e. all protein-coding (1.2%) and non-coding DNA sequences (whole genome sequencing, WGS).[2]

Mutation outside of "classic" hotspots

Retrospective analysis of the PRIME study in patients with metastatic colorectal cancer (mCRC) showed that RAS[a] mutations were not identified in 17% of cases since they lay outside the KRAS[a] mutation hotspot exon 2. This is of great significance for therapy: The exclusion of a RAS[a] mutation is necessary to identify patients who would benefit from targeted EGFR[b] antibody therapy, e.g. cetuximab or panitumumab. NGS-based panel diagnostics now enables assessment of several mutations in a single step.[3]

a (Kirsten) rat sarcoma viral oncogene homolog
b Epidermal growth factor receptor

What is the difference between PCR, IHC and FISH?

PCR:

The polymerase chain reaction (PCR) replicates a specific DNA segment. A few gene copies give rise to millions, which may then be detected and analyzed. RNA may also be analyzed by transcribing RNA into complementary DNA (cDNA) using reverse transcription and subsequent PCR.[1] Primer probes are employed for identification of cancer-specific mutations, such as in NSCLC[c], which are directed against known hotspot mutations in the gene of the EGFR receptor.[4]

IHC:

Immunohistochemistry (IHC) comprises methods which enable identification of certain proteins in tissue sections. This incorporates the use of antibodies which bind to the relevant protein and, by coupling with a dye, provide visual information about the protein quantity of the screened marker.[5]

FISH:

In situ hybridization (ISH) enables detection of specific DNA or RNA sequences. Marked, complementary DNA or RNA probes are also used, which bind to a corresponding DNA/RNA segment in the tissue section.[1] Translocations may thus be detected by removing originally adjacent signals ("break apart" signal), amplifications by comparison of the signal strength of the gene being searched for with a reference marker.[4] The reaction is visible with the fluorescence microscope if fluorescence-marked probes are employed (fluorescence in situ hybridization, FISH).[1]

➔ **Sample application in HER2-positive breast cancer**

Identification of HER2[d] gene amplification using FISH or of HER2[d] protein overexpression using IHC is a prerequisite for targeted therapy with HER2[d]-specific antibodies in patients with invasive breast cancer.[6]

c Non-small cell lung cancer

d Human epidermal growth factor receptor 2

Which clinically relevant insights do panel diagnostics provide?

A large number of genetic mutations associated with cancer are now known thanks to international efforts in sequencing cancer genomes. Extensive genomic data is available for several entities, which enable the development of tumor-specific gene panels for the analysis of individual tumor biology.[7] Depending on the gene panels selected, panel diagnostics enable comprehensive analysis of genetic changes such as[8]

- Single nucleotide variants (SNV)
- Insertions/deletions (indels)
- Change in the number of copies (copy number variation, CNV)
- Chromosomal aberrations such as chromosomal breaks and relocations

The analysis by means of panel diagnostics is not only limited to known hotspots such as oncogenic driver mutations, but allows a comprehensive view of all exons of clinically relevant oncogenes and tumor suppressor genes. Clinically relevant recommendations may be derived by comparing the mutation patterns from tumor biopsies to those stored in databases.[8]

5 applications of panel diagnostics in oncology[1,2]

- Identification of clinically significant gene variants and biomarkers in the tumor
- Estimation of the tumor mutational burden and MSI[e] status
- Identification of appropriate targeted therapy and immunological therapy
- Identification of possible resistance and side effects in patients
- Testing germ line variants relevant for treatment

e Microsatellite instability

Focus on panel diagnostics: 4 reasons for use

Panel diagnostics using NGS-based analysis suffice for most clinical applications in tumor diagnostics. A modest number of target gene regions (ROIs) with known relevance for the appropriate clinical problem are enriched and then sequenced several times over. This allows broad coverage of ROIs* with a large number of individual sequences of each nucleotide within the sequence (coverage). Panel diagnostics offers four advantages in contrast to WES/WGS:[2]

1. It is more cost efficient.
2. It requires a smaller data storage volume.
3. It facilitates raw data analysis (quality control).
4. It facilitates the analysis of the DNA sequence variants and their clinical interpretation.

The disadvantage of panel diagnostics is that genetic changes may also be located outside the selected ROIs. Complex WES/WGS analyses may be useful in such situations.[2] Due to the more complex data analysis, these currently play an important role, especially in research, in better understanding tumor biology, for instance.[8]

* region of interest

Sources

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