Newer Insights in Cancer Molecular Diagnostics

Keyuri Patel, Toshi Mishra, Girish Muzumdar

Cancer is a heterogeneous disease with a medley of molecular alterations. An effective treatment strategy therefore necessitates molecular profiling of tumours for identification of gene expression patterns, which are associated with specific phenotypes and prognosis. It defines a swath of potential targets, which enables one to individualise treatment.

There are two types of biomarkers: prognostic biomarkers which predict patient outcomes and predictive biomarkers, which help in determining response to a certain therapy, in order to maximise treatment benefit but minimise toxicity. A few examples from three common cancers are listed below.

Colorectal Cancer

In advanced colorectal cancer a specific mutation in the KRAS oncogene, is a poor prognostic marker and also a predictive marker, for resistance to anti-epidermal growth factor (EGFR) treatment.[1]

Regarding lung cancer, it is advisable for all patients with advanced non-small cell carcinoma, to undergo biomarker testing.[2]

Advanced Lung Carcinoma

A major breakthrough in treatment of advanced lung adenocarcinoma was the discovery of tyrosine kinase inhibitors (TKI), which target the epidermal growth factor receptor (EGFR). Testing for EGFR gene mutations along with anaplastic lymphoma kinase (ALK) and ROS 1 gene rearrangements as well as programmed cell death 1 ligand 1 (PD-L1) expression, predicts response to EGFR, ALK & ROS 1-targeted inhibitors or immunotherapy, respectively.

Squamous Carcinoma

For squamous carcinoma of lung, PD-L1 immunohistochemistry is useful to select patients who will benefit from immunotheiapy as first line treatment.

Breast Cancer

In case of breast cancer, the disease is classified into five subtypes based on expression of selected marker genes - Luminal A, Luminal B, Human epidermal growth factor receptor (HER 2)-enriched, Basal like, Normal like.[3] Prognosis and therapeutic options differ depending on the subtype e.g. Luminal A subtype will respond to Tamoxifen therapy with good prognosis.

The oncotype DX test is a genomic test, which examines activity of 21 genes in breast tumour tissue to predict likelihood of chemotherapy benefit as well as chance of cancer recurrence in early stage, node negative, ER positive and HER 2 negative disease.

Specimen requirements for molecular testing

Molecular testing can be performed on a variety of clinical samples such as fresh

Dept. of Histopathology, BHIMS, 12 New Marine Lines, Mumbai - 400 020.

/snap frozen tissue, formalin fixed paraffin embedded tissue, cytology specimens, blood, bone marrow and buccal swabs.[4]

Fresh /snap frozen tissue is ideal for studies involving DNA/RNA/protein as it preserves the labile biomolecules in their unaltered forms at the time of sample collection and allows for any type of molecular tests, due to minimal degradation. Formalin-fixed samples are however not suitable for RNA work though they can be used for DNA testing.

Peripheral blood lymphocytes or buccal swabs are used for detection of germ line mutations such as RET mutations in familial medullary carcinoma of thyroid.

Tumour tissue samples are used to detect somatic mutations such as KRAS point mutations in colorectal cancer, SYT /SSX rearrangements in synovial sarcoma and EGFR mutation in lung adenocarcinoma.

Liquid biopsies detect tumour cells and tumour DNA fragments circulating in the bloodstream arising from shedding from primary or metastatic tumour sites. This non-invasive technique uses blood, serum/plasma, urine, CSF and saliva as sample material. It provides a useful alternative for cases when tissue is difficult to obtain, not available, in limited quantity or when the primary site of metastatic disease is unknown.

Molecular profiling techniques

Several techniques such as PCR amplification, Reverse transcriptase-quantitative real time PCR (RT-qPCR), allele specific PCR, DNA sequencing analysis, Immunohistochemistry, Fluorescence/chromogenic in situ hybridisation (FISH /CISH) and DNA microarrays are increasingly being deployed for molecular diagnosis.[4]

Immunohistochemistry (IHC) is a well-established method for identifying both cellular and tissue antigens by means of specific antigen-antibody reactions.

It is a unique tool, which combines molecular detection with morphological features and can be used on formal fixed paraffin sections, frozen sections and cytological smears. The use of IHC in cancer has expanded to involve diagnostic classification, disease prognosis, therapy selection, response prediction, and development of targeted drugs, thus contributing significantly to personalised medicine.

Applications of IHC

For diagnosis and classification

- To differentiate between poorly differentiated carcinomas from lymphoma and melanoma
- To diagnose and further sub-classify haematolymphoid neoplasms
- Diagnosis of malignant round-cell tumours
- To find the probable primary in case of metastatic carcinoma of unknown origin
- To differentiate between adenocarcinoma and mesothelioma
- To classify Neuroendocrine tumours (NET)
- To differentiate between benign / in-

situ and invasive carcinomas in breast (with the help of Myoepithelial markers like Calponin, p63, High Molecular Weight Cytokeratin (HMWCK) etc), prostate (with the help of markers for basal lamina and AMACR).

For prognosis and to predict response to therapy

- To detect micrometastasis in sentinal/regional lymph nodes and bone marrow
- Tumour associated genes like p53
- Growth factor receptors
- Hormone receptors like Oestrogen (OR) and Progesterone (PR) in breast cancers
- C-erbB2 in breast and other cancers like gastric cancer
- Mib-1 labelling index in different cancers like breast etc
- PD-L1 testing in non small cell lung cancer (NSCLC) and melanoma
- P16 as a surrogate marker for HPV in head and neck cancers
- Microsatellite in-stability (MSI) testing colorectal carcinomas

Targeted therapy available for different IHC markers

- For ER-PR in breast cancer: tamoxifen
- C-erbB2 receptor expressed by a subset of breast and other cancers like (gastric): transtuzumab
- C-kit transmembrane receptor in gastrointestinal stromal tumours (GIST): imatinib
- CD 20 expressed by B-cell malignancy: rituximab
- PDL1 protein for both PD-1 and PD-L1 inhibitors in MSCLC and melanoma: pembrolizumab
- ALK expression and EGFR mutation specific antibodies in lung adenocarcinoma
- Screening of hereditary cancer syndromes like Lynch syndrome

Fish is a cytogenetic technique that fluorescent DNA sequence probes that bind to complementary DNA targets on chromosomes. The binding is visualised using fluorescent microscopy. Differentially labelled probes allow one to multiplex for several genes in a sample. This technique reveals presence or absence of specific DNA sequences in cells and tissues e.g. detection of BCR / ALB1 translocation in chronic myeloid leucaemia, Human epidermal growth factor receptor (HER 2) augmentation in breast cancer and ALK rearrangements in adenocarcinoma.

DNA microarray presents a snapshot of the entire transcriptome of a cell in a single reaction. It can be used for whole genome analysis and for chromosome copy number change.

Next generation sequencing (NGS) is a recently evolved DNA sequencing technology that is a high-throughput version of the traditional Sanger sequencing method and uses fluorescence in real time follow sequencing progress. It can rapidly sequence entire tumour genomes or specific genes of interest and discover novel mutations and disease causing genes, due to high sensitivity and large coverage. Precise diagnosis and

classification of disease, more accurate prognosis and identification of mutations which are potentially amenable to drug therapy, have all been rendered possible thereby.[5]

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Axial spondyloarthritis

The term axial spondyloarthritis covers both patients with non-radiographic and radiographic axial spondyloarthritis, which is also termed ankylosing spondylitis. The disease usually starts in the third decade of life with a male to female ratio of two one for radiographic axial spondyloarthritis and of one to one for non-radiographic axial spondyloarthritis. More than 90% heritabilty has been estimated, the highest genetic association being with HLA-B27. The pathogenic role of HLA-B27 is still not clear although various hypotheses are available. On the basis evidence from trials the cytokines tumour necrosis factor (TNF)-? and interleukin-17 appear to have a relevant role in pathogenesis.

MRI being the most important existing imaging method. Non-steroidal anti-flammatory drugs and TNF blockers are effective therapies. Blockade of interleukin-17 is a new and relevant treatment option.

Because the disease affects sacroiliac joints (As opposed to the spine) in most patients, imaging of sacroiliac joints has a pivotal role for diagnosis.

Even in patients with short disease duration (up to 3 years), a definite radiographic sacroiliitis could be seen in 30-50%, which would make further diagnostic procedures unnecessary.

Nonetheless, radiography of sacroiliac joints has limitations in patients with early axial spondyloarthritis, because structural changes generally take months to years to take place. Furthermore, the interpretation of radiographs of the sacroiliac joints is often challenging.

MRI investigation of the sacroiliac joints as the next step.

CT of sacroiliac joints is regarded as a gold standard of structural damage detection (especially erosions), but conventional radiography and MRI of sacroiliac joints usually allow a comprehensive assessment of structural damage. However, CT of these joints (including a so-called low-dose CT, which is associated with a low radiation exposure) might be considered in cases of inconclusive results.

The main treatment target was defined as remission, with low disease activity regarded as a secondary target. For both radiographic and non-radiographic forms axial spondyloarthritis, remission was defined by a low BASDAI score plus normal CRP values or by a low ASDAS, which includes the results of measurements of CRP or erythrocyte sedimentation rate.

Dose reduction or discontinuation should be tried if the patient is in remission.

Joachim Sieper, Denis Poddubnyy, The Lancet, 2017, Vol 390, 73-78