**Cancer Molecular Biomarkers**

 Vallem.Asha

**Sri padmavati mahila visvavidyalayam womens university india**

Abstract :-

Any quantifiable molecular signal of cancer risk, cancer occurrence, or patient outcome is considered a molecular cancer biomarker. Genetic variations in the germline or soma, epigenetic markers, transcriptional modifications, and proteomic markers may all be present. These indications are based on biomolecules that can be found in samples taken from tissues through tumour biopsy or, more conveniently and non-invasively, from blood (or serum or plasma), saliva, buccal swabs, stool, urine, etc. These biomolecules include nucleic acids and proteins. Over the past few decades, detection technologies have evolved significantly, including approaches like next-generation sequencing, nanotechnology, or procedures to examine circulating tumour DNA/RNA or exosomes. There are several clinical uses for biomarkers. They can be utilised as instruments for determining cancer risk, cancer screening and early detection, precise diagnosis, and patient prognosis.cancer surveillance and response monitoring, as well as therapeutic response prediction. As a result, they can aid in improving clinical practise decision-making. Additionally, because recently developed targeted medicines only work in people with particular cancer genetic mutations, precision oncology is required. Biomarkers are the instruments utilised to identify these patient subsets. The scientific hurdle of creating novel biomarkers with higher sensitivity, specificity, and positive predictive value must be overcome, nevertheless, requiring advancement in the field of cancer biomarkers.

Keywords: cancer biomarkers, biomolecules, risk assessment, diagnostic biomarkers, predictive biomarkers

Introduction

A biomarker is a trait that is assessed as a predictor of cancer risk, cancer incidence, or patient prognosis. These traits might be cellular, molecular, physiologic, or image-based. The focus of the current review is on cellular and molecular cancer biomarkers. These biomolecules, which are present or created by cancer cells or healthy cells in response to cancer, can be discovered in tissues or bodily fluids. In order to identify changes in DNA, RNA, proteins, or other biomolecules that can be used for cancer diagnosis, prognosis, precision medicine/guiding cancer treatment, predicting drug response, or cancer monitoring, biomarker testing in cancer entails profiling tumours or bodily fluids. However, genetic testing is used to identify germline genetic variants linked to cancer, which is distinct from cancer biomarker testing.Germline genetic markers can offer important information about therapy alternatives in addition to information on cancer risk . In a larger sense, they can also be thought of as cancer biomarkers. The many biomolecules, sample kinds, and methods used to identify cancer biomarkers are described in the sections that follow. We also go through the various molecular alterations that are helpful as cancer biomarkers. We also go over several cancer biomarker uses in clinical settings as well as the procedures that must be taken from cancer biomarker discovery to clinical application.

**Changes Linked to Cancer:-**

**2.1Variations in Germline Genetics**

There are some hereditary or germline variations that increase the risk of cancer in those who bear them. According to their frequency and ability to spread disease, germline variations can be divided into three categories: rare variants with high penetrance, relatively frequent variants with moderate penetrance, and common variants with low penetrance. Due to their potent effects, the first group, which corresponds to cancer-predisposing syndromes and hereditary malignancies, makes them excellent candidates for use as cancer risk assessment biomarkers. For instance, it is widely known that high-penetrance mutations of BRCA1 and BRCA2 are significantly associated with breast and ovarian cancer. Different cancer types can be affected differently by inherited variations; for instance, Lynch syndrome-associated mutations in the genes EPCAM, MLH1, MLH2, MSH6, and PMS2 have. Furthermore, various genes may have varying cancer risks . Germline genetic markers are significant prognostic and predictive markers for targeted therapy in addition to being helpful for determining cancer risk. Inhibitors of poly (ADP-ribose) polymerase, for instance, are successful in treating germline BRCA mutant breast and ovarian cancer . 853 harmful variants were found and the incidence of pathogenic germline variations in cancer patients was reported to be 8% in a large cancer study with 10,389 cases and 33 cancer types . NGS of the tumor sample can be utilized to identify germline variants in addition to somatic mutations. An FDA-approved NGS panel and pipeline were used in a recent study on more than 21,000 cancer patients, which revealed that tumor-only sequencing missed the majority of germline copy number variations, intronic variants, and repetitive element insertions while correctly identifying 89.5% of pathogenic germline variants . Because germline pathogenic variations have been identified in patients with no family history of cancer, recent investigations have shown the need of examining germline indicators in addition to somatic tumor markers [3]. Additionally, it improves the detection of germline variantsin patients with familial cancer that would not have been discovered otherwise . It also makes variations of unknown importance more prevalent, which can make it challenging to interpret data.

Somatic genetic mutations (2.2).

An essential characteristic of cancer cells is genomic instability, which promotes cancer evolution and allows it to adapt to shifting microenvironments. The majority of cancers are caused by an accumulation of somatic mutations, some of which are unique to a particular cancer type and others of which are shared by several malignancies. The changes might range in size from a single base pair change to a significant portion or entire chromosome. Chromosomal abnormalities include structural (translocations, inversions, copy number variations, including insertions and deletions, as well as chromothripsis, which canlead to significant rearrangements) and numerical abnormalities (aneuploidies and polyploidies). Relevant cancer biomarkers are those particular and recurrent chromosomal abnormalities, and they are primarily used in hematopoietic malignancies. For instance, theThe Philadelphia chromosome, which involves the translocation of chromosomes 22 and 9, which results in BCR-ABL fusion, was the first chromosomal aberration to be identified in cancer. It is frequently observed in chronic myeloid leukemia but can also occur in acute myeloid leukemia and is utilized to diagnose both of these conditions. a thorough list of gene fusions discovered through examination of RNA sequencing and DNA. However, the more common genetic alterations used as cancer biomarkers are mutations involving a single nucleotide (single nucleotide variants or SNVs) or a few nucleotides (small insertions and deletions or indels), e.g., driver mutations in EGFR, KRAS, BRAF, TP53, KITK, and other genes. Results from the Pan-Cancer Gene Atlas sequencing project [[9](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9331210/#B9-biomolecules-12-01021)] on 3281 tumors from 12 cancer types identified 127 recurrently mutated genes. The mutation frequency was found to depend on tumor types ranging from 0.28 mutations/Mb in acute myeloid leukemia to 8.15 in lung squamous cell carcinoma. Despite the large number of mutations seen in each tumor, only four or five mutations are thought to be drivers of cancer development [[10](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9331210/#B10-biomolecules-12-01021)]. A more recent and detailed analysis of Pan-Cancer data from 33 different cancer types identified 299 driver genes and around 3400 driver mutations based on in silico methods together with experimental validation [[11](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9331210/#B11-biomolecules-12-01021)]. Mutations in TP53 were found to be the most commonly shared among 27 different cancer types, followedby PIK3CA, KRAS, PTEN, and ARID1A (in 15 or more). However, the majority of the genes (142) were found mutated in only one cancer type. Interestingly, they reported that 57% of tumors had mutations that data from 33 distinct sources on pan-cancerA more recent and thorough examination of Single nucleotide variants, or SNVs, or tiny insertions and deletions, or indels, such as driver mutations in the EGFR, KRAS, BRAF, TP53, KITK, and other genes, are the genetic changes that are employed as cancer biomarkers most frequently.127 recurrently altered genes were found in 3281 tumors from 12 different cancer types as a result of the PanCancer Gene Atlas sequencing project  results.The frequency of mutations was discovered to vary according on the type of tumor, ranging from 8.15 for lung squamous cell carcinoma to 0.28 mutations/Mb in acute myeloid leukemia.Only four or five mutations are believedto be the main initiators of cancer formation, despite the high number of mutations observed in each tumBased on in silico techniques and experimental confirmation, y cancer types identified 299 driver genes and over 3400 driver mutations . The majority of 27 different cancer types were discovered to share TP53 mutations, followed by PIK3CA, KRAS, PTEN, and ARID1A (in 15 or more). However, only one kind of cancer was discovered to have mutations in the majority of the genes (142).

2.3. Epigenetic Variants

Only four or five mutations are believed Epigenetic variants cause changes in DNA methylation or histone protein modifications, without affecting the coding sequence of DNA. However, they affect the structure and stability of DNA and play an important role in carcinogenesis. These epigenetic changes in cancer cells are therefore useful as cancer biomarkers, especially since DNA methylation changes occur in the early stages of tumorigenesis . Loss of global DNA methylation is common in many tumors and is associated with genomic instability, DNA damage, and reactivation of transposons and retroviruses. Moreover, more localized changes in DNA methylation at the CpG-rich promoter regions of the genes can inactivate tumor suppressor genes. For example, the CpG island methylator phenotype, characterized by hypermethylation of multiple sites, is a feature associated with genomic instability and cancer . Methylation of CACN3A1G, IGF2, NEUROG1, RUNX3, and SOCS1 promoters is common in this phenotype and is associated with MLH1 methylation and microsatellite instability. It is noteworthy that MLH1 methylation is a biomarker used for cancer-prone Lynch syndrome testing in clinics . In addition, DNA methylation biomarkers are also useful for predictsto histone proteins or DNA methylation. However, they have an impact on DNA's stability and structure and are crucial in the development of cancer. Because DNA methylation modifications take place in the early phases of carcinogenesis, these epigenetic changes in cancer cells are therefore helpful as cancer biomarkers. Genomic instability, DNA damage, and the reactivation of transposons and retroviruses are all linked to loss of global DNA methylation, which is prevalent in many cancers. Additionally, tumor suppressor genes can be rendered inactive by more focused modifications in DNA methylation in the CpG-rich promoter regions of the genes. For instance, the CpG island methylator phenotype, which is defined by numerous sites being hypermethylated, is linked to chromosomal instability and cancer.

### 2.4. Transcriptional Alterations

Both messenger or coding RNA (mRNA) and non-coding RNA (ncRNA) are present in the human transcriptome. Small ncRNAs include microRNA (miRNA), small interfering RNA (siRNA), small nucleolar RNA (snuRNA), ribosomal RNA (rRNA), transfer RNA, and piwi-interacting RNA. Long ncRNA (lncRNA) is the largest subgroup of ncRNA and includes long intergenic RNA, antisense RNA, pseudogenes, and circular RNAs (circRNA). It has been discovered that coding and non-coding RNAs are differently expressed in cancer and are important in the development of the disease. Additionally, some of these ctRNA molecules—which stand for cell-free RNA molecules—can be discovered.

**Molecular cancer biomarker sources**

Tumor tissue has been the subject of the most extensive examination to far, however cancer biomarkers can be examined from a range of material types. Liquid biopsies, which are typically non-invasive, are an alternative to tumor biopsies. Blood, urine, stool, and, less frequently, exhaled breath, saliva/buccal swabs, cerebrospinal fluid, sputum, and other bodily fluids are the non-tumor sample types used for cancer biomarker analysis.

### Techniques Used to Detect Molecular Cancer Biomarkers

### FISH

### It uses a fluorescently labeled probe that hybridizes with DNA to find gene fusions or changes in gene copy number in tumor cells or sections (for example, HER2 amplification). Multiplex FISH, spectral karyotyping, and comparative genomic hybridization are a few FISH variations. Spectral karyotyping is a 24-color chromosome painting test that has a high sensitivity for detecting chromosomal abnormalities. Particularly in hematological malignancies, sarcomas, carcinomas, and brain tumors, it can be employed to find chromosomal biomarkers of cancer diagnosis and prognosis .

### PCR/Real-Time PCR/Digital PCR

PCR-based targeted genetic profiling is the most common technology used in cancer diagnostics for both DNA- and RNA-based applications. It is used for the detection of small DNA mutations (e.g., EGFR mutations), gene fusions (e.g., RNA-based testing for ALK), or DNA methylation analysis using methylation-specific PCR (e.g., MGMT promoter methylation in glioblastoma or Septin9 gene methylation in CRC). Many modifications of this basic method are continuously being developed to increase the sensitivity of detecting biomarkers from trace sources.

### NGS

NGS is finding application in genetic screening of both germline variants and somatic mutations, including SNVs, indels, and CNAs. It is also being used for RNA-based biomarkers, such as gene fusions and RNA sequencing. The approaches include both amplicon-based screening using primer panels to amplify regions of interest harboring driver gene mutations, or targeted capture and hybridization for selecting fragments of interest for sequencing using capture probes. Different kinds of NGS gene panels have been developed: cancer-specific panels (e.g., for lung cancer, CRC, and breast cancer), general pan-cancer panels for solid tumors or hematological cancers, or panels designed to detect genomic changes for targeted therapies. Noteworthy, NGS-based tests are sensitive to the platform and methods used, and they are therefore mainly approved for testing at a specific site. Other challenges in NGS-based methods relate to differentiating cancer driver mutations from passenger mutations and setting a minimum threshold mutant allele frequency for variant calling.

### Flow Cytometry

It is often applied in leukemia and lymphoma diagnostics to identify and count cells by using a panel of fluorescently labeled antibodies. It is also deployed to quantitate DNA in cancer cells by treating them with DNA-binding, light-sensitive dyes. Changes in DNA quantity indicate cancer recurrence in breast, prostate, or bladder cancer. What is more, it has application in CTC-based biomarkers, as well.

### CRISPR-Based ctDNA/RNA Detection

Another promising technique that could simplify the detection of ctDNA/RNA and increase its sensitivity and specificity has been recently developed based on clustered regularly interspaced short palindromic repeats (CRISPR) technology. It is known that different CRISPR-associated (Cas) enzymes can be used to detect different nucleic acids. Therefore, by combining an RNA-guided RNase Cas13a, a target-specific CRISPR RNA, and a labeled reporter RNA, RNA signals can be detected without the need for nucleic acid amplification steps. Interestingly, the CRISPR/Cas13a system integrated into microfluidic chips with biosensors has been successful in detecting miRNA biomarkers in serum samples of brain cancer patients with remarkable sensitivity of detection (10 pM in a volume of less than 0.6 µL and in less than 4 h of processing time) . Similarly, an assay using the CRISPR-Cas14a system and strand displacement amplification for detecting miR-21 expression in blood was shown to discriminate cholangiocarcinoma patients from controls.

### Clinical Applications of Cancer Biomarkers: Examples

### The clinical applications of cancer biomarkers are extensive, and their ultimate goal is to achieve precision medicine to optimize prevention, screening, and treatment strategies of cancer. These applications include risk assessment; screening and early detection; accurate diagnosis; patient prognosis; prediction of response to therapy; and cancer surveillance and monitoring response.

### Cancer Risk Assessment Biomarkers

A cancer risk or susceptibility biomarker is used to identify individuals with a higher probability to develop cancer compared to the standard risk in the general population. Cancer risk biomarker tests include DNA repair phenotype assays, as well as genotyping assays for germline variants. DNA repair has shown clear interindividual variability related to cancer susceptibility . Several technologies have been reported to quantify DNA repair capacity (and also DNA damage and DNA damage response): comet assay, ɣH2AX foci formation, host cell reactivation assay, DNA repair beacons, and others (reviewed in Reference . However, in the last decades, genotyping assays have gained importance thanks to the development of high-throughput NGS tools. In a recent study performed with a large multicenter cohort, the personal risk for hereditary cancer syndromes, among other disorders, was evaluated in healthy individuals . Disease-predisposing variants related to cancer syndromes were present in 7.7% of individuals analyzed. Clinically significant variants were commonly detected in MUTYH, CHEK2, APC, ATM, BRCA1, BRCA2, MITF, HOXB13, PMS2, PALB2, NBN, BRIP1, MSH6, SDHA, and BARD1. These findings would prove the utility of using genetic screening as part of regular medical care , althoughthere are doubts about the interpretation of variants of uncertain effect.

### Screening and Early Cancer Detection Biomarkers

The purpose of these biomarkers is to detect cancer in otherwise healthy patients, and without having shown any signs of disease. Actually, they are suggesting the presence of cancer, which has to be diagnosed with other medical approaches. The main justification for their use is the fact that if cancer is detected in an early and asymptomatic stage, the survival rate increases, and the probabilities of complications or morbidities decrease. However, in some cases, the use of these biomarkers leads to overdiagnosis, that is, detection of a cancer that would never cause any symptoms.

### Accurate Cancer Diagnosis Biomarkers

Diagnostic biomarkers are used to confirm the presence of cancer or to identify a subtype of cancer. The usefulness of these biomarkers is that proper diagnosis can lead to proper treatment and, therefore, best chances of survival. Some screening biomarkers are also used as diagnostic biomarkers; however, the latter is only applied to symptomatic patients, whereas screening biomarkers are applied to asymptomatic individuals. Despite diagnostic biomarkers can help to detect cancer or to classify patients into subtypes, they are not sufficient for a final diagnosis and need to be combined with other diagnostic procedures, such as imaging or biopsies.

### Patient Prognosis Biomarker

Once a tumor has been diagnosed, a prognostic biomarker provides information about the probable course of the disease, including its recurrence, progression, and patient’s overall survival, regardless of the treatment. Sometimes, these biomarkers can reflect tumor burden, and then they can help in determining the stage of cancer (e.g., the tumor–node–metastasis classification). Some examples of prognostic biomarkers widely used are protein biomarkers, such as CEA for CRC, CA19-9 for pancreatic cancer, and CA125 for ovarian cancer; and some tests based on gene expression signatures for breast cancer, such as MammaPrint and Prosigna Other examples would be the genetic alterations that facilitate an accurate risk-stratification of patients with acute leukemia that are associated with patient outcomes .

### Biomarkers Predicting Response to Cancer Therapy

It is well-known that treatment decisions are critical in cancer patient management, and often there is uncertainty in the levels of response, as biomarkers are increasingly playing a key role in the optimization of cancer treatment, based on the idea that specific tumor alterations or specific germline genetic variants (pharmacogenetics) yield a certain pattern of sensitivity to cancer therapy agents.Predictive biomarkers aim to estimate the effect of a specific therapy on a cancer patient before treatment has started. According to the results of the biomarker assay, cancer patients can be classified as probable responders or non-responders to a specific therapy, either chemotherapy, endocrine therapy, radiotherapy, or the emerging targeted strategies and immunotherapy. Some of these biomarkers can also identify those patients that will likely show severe toxicity after therapy. Predictive biomarkers can be very useful for adjusting treatment doses or guiding alternative therapies in patients classified as non-responders or with a high risk of toxicity.

### Biomarkers for Cancer Surveillance and Monitoring Response

A monitoring biomarker is assessed serially over time, e.g., during treatment with curative intent or after it has finished. This can allow for comparisons to observe, for example, real-time overall disease burden, to detect worsening of the disease, or to follow disease response to treatment. Liquid-biopsy-based biomarkers are the best option for minimal residual disease monitoring and cancer surveillance. In this sense, several studies have reported ctDNA as a promising good monitoring biomarker, since it is believed that ctDNA levels correlate with tumor burden over time. Therefore, monitoring ctDNA in cancer patients could help to detect early recurrences or residual cancer that would otherwise remain undetected by other methods, such as imaging.

### Conclusions and Future Perspectives

### Cancer cells undergo multiple changes, and these alterations have been used for decades as cancer biomarkers, mainly tested in tumor tissue. Recent research in the cancer biomarker field has helped in the development of new DNA, RNA, and protein-based cancer biomarkers that can be detected from easily available body fluids. NGS has opened up possibilities for analyzing all cancer-associated genetic alterations in a single assay. Moreover, increased benefits of including analysis of both germline and somatic mutations in a single panel are being recognized for precision oncology. However, most NGS-based tests are optimized for panel, sequencing platform, and site. In addition, although high-throughput transcriptomic and proteomic studies have identified new candidate cancer biomarkers, only very few have been clinically implemented. The biggest challenge in cancer biomarker detection from body fluids is their very low concentration. To overcome this, highly sensitive detection technologies are being developed. For example, nanoparticles with a high surface-to-volume ratio make it possible to attach different molecules to their surface; this, combined with sensor technology, for signal amplification and detection, offers opportunities for the development of more sensitive cancer biomarkers from the body fluids. Finally, it is important to consider all the steps needed to develop a new biomarker. Before clinical implementation, requirements related to analytical and clinical validation, as well as clinical utility, must be fulfilled in order to obtain the necessary regulatory approval by authorities.

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