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Molecular Diagnostics in Oncology: Promising Better Clinical Outcomes with Patient-centric Approach

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Abstract

Molecular diagnostics play a crucial role in oncology by analyzing DNA, RNA, and proteins to improve cancer detection, guide targeted therapy selection, and monitor disease progression. This approach enables personalized medicine by identifying specific genetic and molecular alterations, which helps clinicians provide more precise treatments and potentially improve patient outcomes. Molecular diagnostics play a pivotal role in modern oncology by enabling precise identification of hereditary cancer syndromes, guiding preventive care for mutation carriers, and informing tailored treatment strategies. Mutation profiling supports the use of targeted therapies such as EGFR, BRAF, ALK, ROS1, and PARP inhibitors. Liquid biopsy techniques—detecting tumor-derived DNA, RNA, or proteins in body fluids—offer non-invasive options for disease monitoring, early detection, and drug sensitivity analysis. Additionally, molecular markers aid in diagnosing cancers of unknown primary origin, while systematic tumor profiling continues to uncover novel DNA- and RNA-based biomarkers with clinical relevance.

Introduction

In the modern era molecular diagnostics forms the backbone of so-called “personalized” or “precision” medicine, is a tailored approach to the treatment to individual patients based on molecular insights. Precision medicine, is a revolutionary and a relatively new approach in healthcare that acts as transformative healthcare paradigm that leverages molecular data to guide personalized treatment strategies. [1, 2]

Molecular diagnostics include tests that can detect genetic material, proteins, or related molecules that provide information about health or disease. By analyzing DNA, RNA, proteins, and other biomarkers, clinicians can make informed decisions that optimize therapeutic outcomes. These tests commonly utilize samples of blood, saliva, or tumor tissue. Depending on the type of test, a molecular diagnostic test includes gene panels, gene expression profiles, and gene signature panels—each offering unique insights into disease mechanisms and therapeutic targets. [2]

Molecular diagnostics enables early detection, accurate diagnosis, and targeted therapy selection, facilitates risk stratification and monitoring of treatment response, and supports the development of companion diagnostics for novel therapeutics. [1,2]

Challenges in Oncology Molecular Diagnostics

Cancer is an inherently complex disease, characterized by multistage progression and significant heterogeneity. This variability arises from a dynamic interplay of genetic mutations and epigenetic modifications, making early detection and monitoring particularly difficult. Existing gold-standard

diagnostic modalities often include invasive issue biopsies that carry risks such as bleeding, infection, and sampling bias. Besides, cancer diagnosis is often delayed as many cancers remain asymptomatic till advanced stages, reducing therapeutic efficacy and survival rates. This necessitates the introduction and utilization of noninvasive diagnostic methods with high accuracy, safety and early detection of the disease. [3]

Prioritizing innovative diagnostic modalities to overcome these limitations, the field demands:

- **Noninvasive Modalities**: Methods such as liquid biopsy, circulating tumor DNA (ctDNA) analysis, and exosomal profiling. [3]
- **High Sensitivity and Specificity**: Tools that can detect molecular alterations at the earliest stages with negligible false positives.[3]
- **Real-Time Monitoring**: Dynamic assays that track disease progression and treatment response longitudinally. [3]

Applications of Molecular Diagnostics in Clinical Oncology

Molecular Risk Assessment [2]

Molecular diagnostics are progressively used to evaluate an individual's predisposition to specific cancers- the approach is often termed molecular profiling or molecular risk assessment. An example of a molecular diagnostic test used for risk assessment is the blood test for the pathogenic variants in breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2) genes. Alterations in either of these genes can increase the lifetime risk of breast, ovarian, and several other cancers. [2]

Differential Diagnosis of a Specific Type of Cancer

Molecular diagnostics play a pivotal role in: differentiating malignancy from benign tumors, identifying the tissue of origin in cancers (e.g., breast, lung, skin, etc.), and classifying different cancer subtypes within the single cancer type/same tissue. For example, there are many different types of blood cancers. Molecular diagnostics are widely used for risk stratification. For example; a type of blood cancer commonly known as acute myelogenous leukemia (AML), can be classified in one of three risk categories: poor, intermediate, or favourable risk based on cytogenetic analysis. [3] Moreover, as per World Health Organization (WHO) and in the National Comprehensive Cancer Network (NCCN) guidelines molecular testing is considered as standard of care in intermediate-risk AML patients. Molecular diagnostics can aid in prognosis and thereby guiding clinicians' decision regarding therapy. For example, genetic abnormalities (chromosomal abnormalities and mutations) in proliferation/survival mechanisms (Fms-like tyrosine kinase 3-FLT3) and differentiation/apoptosis pathways (CCAAT/enhancer-binding protein alpha (CEBPA), Runt-related transcription factor 1(RUNX1, and Nucleophosmin 1 (NPM1), play a key role in the pathogenesis of AML, which provide prognostic criteria, and can guide therapy. WHO) and NCCN guidelines recommend it as a standard of care molecular testing. Acute promyelocitic leukemia (APL), one subtype of AML, has specific molecular markers such as Promyelocytic Leukemia–Retinoic Acid Receptor Alpha (PML-RARA) associated with it. Molecular analysis of PML-RARA in APL is now standard of care for several different mutations. [5]

In chronic myeloid leukemia (CML), a specific chromosomal translocation of chromosomes 9 and 22 occurs, which is known as the Philadelphia chromosome. This characteristic chromosomal abnormality can be detected by cytogenetics, by fluorescence in situ hybridization (FISH), or by polymerase chain reaction (PCR) targeted at the breakpoint cluster region gene and the Abelson gene (BCR/ABL) fusion gene. [3] The presence of

this fusion gene is crucial for the diagnosis and management of CML and certain types of acute leukemia, often targeted by BCR/ABL tyrosine kinase inhibitors (TKIs). [6]

Prognostic Significance

Prognosis refers to the prediction of the anticipated course and outcome of a disease or injury, including the likelihood of recovery. It is influenced by multiple factors such as type and severity of disease or injury, patient-specific variables (age, sex, race), treatment modalities and timing, and biological behaviour of the condition (e.g., aggressiveness of cancer). One of the most clinically notable examples is the mutation of the FLT3 gene which is the most common genetic alteration and a poor prognostic factor in AML patients. The detection of FLT3 alterations by a molecular diagnostic (PCR & next- generation sequencing) might prove beneficial in predicting treatment response. [7] Molecular diagnostics can also be used to determine the probability of cancer recurrence after treatment—another aspect of prognosis. Several molecular diagnostics are available to predict the chances of breast cancer recurrence in women with early-stage, node-negative, estrogen receptor-positive, invasive breast cancer who will be treated with hormone therapy. [2]

Prediction of Therapeutic Response

Molecular diagnostics can help in predicting therapeutic/treatment response in cancer patients. An example is the test for human epidermal growth factor receptor 2/neu (HER2/neu) gene overexpression in breast cancer tumor tissue. The HER2/neu gene regulates the synthesis of HER 2 proteins. Around 25% of all breast cancers have abundant copies of this gene and so synthesis of too much protein. The extra protein causes HER-2 positive breast cancer. A monoclonal antibody trastuzumab used as a therapeutic agent for HER-2 positive breast cancers inhibits the activity of the HER2 protein. Besides, testing for HER2/neu can aid in determining aggressiveness of breast cancer which has prognostic value. [2]

Pharmacokinetics

Pharmacokinetics encompasses drug absorption, distribution, metabolism, and elimination by the body. Genetic differences between individuals affect the rate of occurrence of these processes due to which rapid metabolization of drugs in some people compared to others which has major implications for cancer therapies. The use of multi-gene testing assays for individualized treatment strategies is rapidly emerging into routine oncology practice. UGT1A1 gene encoding for the uridine diphosphate glucuronosyltransferase (UGT) 1A1 enzyme, is usually utilized for multi-gene molecular testing platforms. UGT1A1 gene polymorphisms might influence drug-induced toxicities of many medicines used in oncology. [8] For example, irinotecan drug is used for the treatment of colon cancer; in individuals with a genetic pattern designated UGT1A1*28 irinotecan metabolism is slow compared to those without this pattern. Therefore, to prevent drug-induced toxicity due to accumulation of drug in the body, a lower dose than normal must be administered to these individuals. [2]

Monitoring Treatment Response/Course of Treatment

In colon cancer molecular diagnostics can be repeated multiple times during the course of treatment to inspect treatment response. Some cancers such as CML can develop resistance to medications and in this scenario a novel resistance mutation should be identified swiftly to switchover on to a different TKI therapy that could inhibit the ABL protein concealing the mutation thereby preventing the emerging resistant clone. NGS is potentially beneficial in identifying the presence of a mutation within ABL. [9]

Table 1: Molecular Diagnostics in Cancer [2]

Molecular test	Type of Cancer
HPV	Cervical cancer
BRACA1 & BRCA2 genes	Breast, Ovarian and Cervical cancers
Cancer Antigen 125 (CA125) protein	Ovarian cancer
PSA	Prostate cancer
HER2/neu gene	Breast cancer
DNA mismatch repair genes (MSH2, MLH1, MSH6, PSM2)	Colon cancer (Lynch syndrome)
KIT gene	AML and GIST tumors of gastrointestinal tract
EGFR, ALK and HRAS genes	Lung cancer
HPV: Human Papilloma Virus; **BRACA 1& BRACA 2:** Breast Cancer Gene 1& Breast Cancer Gene 2; **PSA:** Prostate Specific Antigen; **HER2/neu gene:** Human Epidermal Growth Factor receptor 2; **MSH2:** mutS homolog 2; **MLH1:** mutL homolog 1; **MSH6:** mutS homolog 6; **PMS2:** postmeiotic segregation increased 2; **KIT:** Proto-oncogene, receptor tyrosine kinase; **EGFR:** Epidermal Growth Factor Receptor; **ALK:** Anaplastic Lymphoma Kinase; **HRAS:** Harvey Rat Sarcoma Viral Oncogene Homolog;	

Types of Genetic Alterations in Cancer

Mutations in the genes encoding cellular signaling molecules, particularly protein kinases, can lead to cancers. Therapeutic treatments targeting mutant protein kinases can prove efficacious in cancer patients. The sensitivity of these drugs is linked to the genetic composition of individual tumors. Therefore, mutational profiling of tumor DNA aids in prioritizing patient-centric anti-cancer treatment strategies. [10]

Alterations/Mutations in Genotype: Four main types of genetic alterations result in cancer which are as discussed below: [10]

Point mutations-Single nucleotide variants (SNVs) - point mutations

SNVs arise from the substitution of a single nucleotide base in the DNA sequence. These alterations can lead to:

- **Missense mutations**: A codon change by the incorporation of a different amino acid, potentially altering protein function. [10]
- **Nonsense mutations**: A codon change by the introduction of a premature stop codon, leading to truncated, usually nonfunctional proteins. [10]

Frameshift Mutations -Insertions or deletions

Insertions and deletions (indels) involving one or a few nucleotides can disrupt the DNA coding sequence in two major ways:

- **In-frame mutations [10]**
  - Indels in multiples of three nucleotides.
  - Preserve the reading frame.
  - Result in addition or removal of amino acids without truncating the protein.
- **Frameshift mutations [10]**
  - Indels not in multiples of three.
  - Shift the reading frame downstream.
  - Often introduce premature stop codons, producing truncated and typically nonfunctional proteins.

Exon or gene copy number changes

Copy number variations (CNVs) refer to duplications or deletions of DNA segments that can significantly impact gene function and expression. [10]

- **Exon-level CNVs [10]**
  - Involve large duplications or deletions of one or more exons.
  - May disrupt functional protein domains, altering protein activity or stability.
- **Gene-level CNVs [10]**
  - Include amplification or deletion of entire genes.
  - Can lead to overexpression of oncogenes or loss of tumor suppressor function.

Structural variants (SVs) or large structural anomalies

Structural variants are large-scale genomic alterations involving rearrangements of DNA segments. These include:

- **Translocations**:
  - DNA segments are exchanged between different chromosomes. [10]
  - Often result in fusion genes (e.g., BCR-ABL) that encode oncogenic fusion proteins (Box 1). [10]
- **Inversions**:
  - DNA segments are reversed within the same chromosome. [10]
  - May disrupt gene integrity or regulatory elements. [10]

BOX 1: Philadelphia chromosome- A classic example of translocation-associated malignancy

CML originates when the fusion of portion of chromosome 9 sheltering the gene coding for the tyrosine-protein kinase, Abelson murine leukemia (ABL), occurs with the gene of the breakpoint-cluster region (BCR) protein located on chromosome 22 forming the *BCR-ABL* fusion gene (commonly known as the Philadelphia chromosome). The BCR-ABL fusion protein, displays augmented tyrosine kinase activity and is the target of molecularly targeted cancer treatments. [11]

Alterations in Phenotype (Epigenetics)

Epigenetics refers to heritable changes in gene expression and chromatin architecture that occur independently of alterations in the DNA sequence. These modifications regulate cellular phenotype without modifying genotype, and are central to differentiation in multicellular eukaryotes—excluding the antigen receptor diversification in T- and B-cell maturation. [12]

Key epigenetic mechanisms include:

- **DNA and RNA methylation**: Typically silences gene expression via methyl groups added to cytosine residues or RNA bases. [12]
- **Histone modifications**: Acetylation, methylation, phosphorylation, and ubiquitination alter chromatin accessibility and transcriptional activity. [12]
- **MicroRNA (miRNA) expression**: Post-transcriptional regulation through RNA interference pathways. [12]

Cancer Biomarkers

Definition of Biomarkers

National Cancer Institute (NCI) define biomarkers as “a biological molecule present in blood, other body fluids, or tissues encoding about a normal or abnormal process, or of a medical condition or disease”, such as cancer. Biomarkers help in differentiation of healthy individuals from affected ones and can arise due to variables such as germline or somatic mutations, transcriptional changes, and posttranslational modifications. [13]

Biomarkers may include proteins (e.g., enzymes or receptors), nucleic acids (microRNA or other non-coding RNA), antibodies, peptides, composite molecular patterns (e.g., gene expression, proteomic, metabolomic signatures). Clinically, biomarkers are used to predict prognosis, estimate recurrence risk, and guide treatment decisions—often without initiating therapy. [14]

Types of Cancer Biomarkers

Genetic Biomarkers [15]

Cancer biomarkers span molecular, cellular, and metabolic domains, offering diagnostic, prognostic, and therapeutic insights. [15] They are broadly categorized as follows:

Deoxyribonucleic Acid (DNA) as a Cancer Biomarker

Elevated serum DNA levels correlate with metastatic disease. Mutations in oncogenes, mismatch-repair genes, and tumor suppressors such as BRCA1 serve as key biomarkers. [15]

RNA as a Cancer Biomarker

RNA expression profiling has emerged as a powerful approach for identifying cancer biomarkers. Techniques such as differential display, reverse transcription quantitative PCR (RT-qPCR), bead-based multiplex assays, and microarray analysis are routinely employed to detect and quantify RNA-level alterations. Tumor microRNA profiles can be used to identify important subgroups, survival rates, and therapy response. For instance, in chronic lymphocytic leukemia (CLL), the co-expression of miR-15 and miR-16 has been linked to disease pathogenesis and progression. [15] Similarly, elevated levels of miR-483-3p have been reported in pancreatic ductal adenocarcinoma (PDAC) compared to healthy controls ($p < 0.01$), suggesting its potential utility as a non-invasive diagnostic marker. [16]

Epigenetics as a Cancer Biomarker

Epigenetic alterations, particularly DNA methylation, represent robust biomarkers for cancer due to their gene-specific frequency, biological stability, and compatibility with minimally invasive detection methods. Aberrant activity of DNA methyltransferases, which catalyze the addition of methyl groups to cytosine residues, is frequently observed in malignant cells. Blood-based assays utilizing cell-free DNA (cfDNA) have enabled the non-invasive characterization of epigenetic changes in tumor suppressor genes, offering a promising adjunct to conventional screening modalities such as mammography. One of the most common examples is promoter hypermethylation of cytosine-phosphate-guanine (CpG) islands is a well-established mechanism for silencing tumor suppressor genes, thereby contributing to oncogenesis. For example, methylation of NK2 homeobox 6 (NKX2-6), Sperm Associated Antigen 6 (SPAG6), period circadian regulator 1 (PER1), and inter-alpha-trypsin inhibitor heavy chain 5 (ITIH5) has been detected in the serum of breast cancer patients. [17] Similarly, hypermethylation of the p16 promoter in cfDNA is associated with recurrent colorectal cancer. Early screening for colorectal cancer has also leveraged methylation markers such as MYCN proto-oncogene (N-Myc), downstream-regulated gene-4 (NDRG4), and bone morphogenetic protein-3 (BMP3). [15] DNA methylation biomarkers like N-Myc, downstream-regulated gene-4 (NDRG4) and bone morphogenetic protein-3 (BMP3), both tumor suppressor genes, can be used for early CRC screening. In lung cancer, methylation of Ras association domain family 1 isoform A (RASSF1A) and cyclin-dependent kinase 4 inhibitor A (p16Ink4) correlates with a 15-fold increase in relative risk. [15] Ovarian cancer studies have reported promoter h)) hashylation of RASSF1A and BRCA1 in 68% of tumor tissues, and a five-gene cfDNA methylation panel (BRCA1, hypermethylated in cancer 1(HIC1), paired box 5 (PAX5), progesterone

receptor (PGR), thrombospondin 1 (THBS1)) has demonstrated 85% sensitivity and 61% specificity for detection These findings underscore the utility of multiplexed methylation profiling in cfDNA as a liquid biopsy strategy, enhancing early detection and risk stratification across multiple cancer types. [19]

5.1.1 Protein as Cancer Biomarkers

Table 2: Various Protein Biomarkers used in Cancer Detection

Biomarker	Associated cancer(s)	Clinical utility	Sample type
AFP [15, 20]	Liver, testicular, ovarian	Diagnosis, staging, monitoring	Serum
CEA [15, 20]	Colorectal, pancreatic, breast, lung	Prognosis, recurrence monitoring	Serum
PSA [15, 20]	Prostate	Screening, diagnosis, treatment response	Serum
CA-125 [15, 20]	Ovarian	Diagnosis, monitoring, recurrence detection	Serum
CA19-9 [15, 20]	Pancreatic, colorectal	Diagnosis, prognosis, treatment monitoring	Serum
CA15-3 [15, 20]	Breast	Monitoring recurrence and treatment response	Serum
CA27.29 [15, 20]	Breast	Similar to CA15-3; used in recurrence monitoring	Serum
hCG [15, 20]	Testicular, gestational trophoblastic	Diagnosis, staging, treatment monitoring	Serum, urine
HER2 [15, 20]	Breast, gastric	Prognosis, targeted therapy selection	Tissue (IHC/FISH)

Metabolic Biomarkers

Metabolites and metabolic pathways are fundamentally altered in cancer cells to sustain rapid proliferation, evade apoptosis, and adapt to hostile microenvironments. These metabolic reprogramming events—hallmarks of malignancy—generate distinct biochemical signatures that serve as valuable biomarkers for cancer detection, prognosis, and therapeutic monitoring. [15]

Glycolysis

One of the hallmark metabolic alterations in cancer is the shift toward aerobic glycolysis, commonly referred to as the Warburg effect. Key biomarkers associated with glycolytic activity include:

- **Lactate**: Elevated lactate concentrations in tumor tissues or serum reflect enhanced glycolytic flux and are indicative of aggressive tumor phenotypes. [15]
- **Glucose Transporters (e.g., GLUT1)**: Overexpression of glucose transporters, particularly GLUT1, facilitates increased glucose uptake and is frequently observed across multiple cancer types, correlating with poor prognosis and therapeutic resistance. [15]

TCA Cycle (Tricarboxylic Acid Cycle)

Alterations in the TCA cycle are increasingly recognized as hallmarks of cancer metabolism, with several intermediates serving as potential biomarkers. Notably:

- **Fumarate and Succinate**: Accumulation of these metabolites is linked to loss-of-

function mutations in fumarate hydratase (FH) and succinate dehydrogenase (SDH), respectively. [15]

- **α-Ketoglutarate (α-KG)**: Dysregulated α-KG levels have been observed in various malignancies, including renal cell carcinoma. Altered α-KG metabolism can influence histone and DNA demethylation, impacting gene expression and tumor progression. [15]

Lipid Metabolism

Altered lipid metabolism is a hallmark of cancer, supporting membrane synthesis, energy production, and signaling pathways essential for tumor growth and survival.

- **Choline**

Elevated choline levels are consistently observed in malignancies such as breast and prostate cancers. [15]

- **Fatty acid synthase (FASN)**

Various cancers display the overexpression of FASN enzyme that is involved in fatty acid synthesis biochemical pathway. [15]

Amino Acid Metabolism

Cancer cells undergo profound reprogramming of amino acid metabolism to support their biosynthetic demands, redox balance, and epigenetic regulation. These alterations yield distinct metabolic signatures that serve as potential biomarkers and therapeutic targets. [15]

Key examples include:

- **Glutamine**

Increased glutamine uptake and utilization are observed across multiple tumor types, including glioblastoma, pancreatic, and lung cancers. [15]

- **Serine and Glycine**

Dysregulated metabolism of serine and glycine—critical for one-carbon metabolism and nucleotide biosynthesis—is frequently seen in breast and colorectal cancers. [15]

Nucleotide Metabolism

Biomarkers of nucleotide metabolism include deoxythymidine (dThd). Elevated concentrations of dThd have been reported in several malignancies and can be detected in biofluids such as urine and plasma. [15]

Cells as Cancer Biomarkers

Circulating cells—including cancer cells, immune cells, and stem-like tumor cells mostly emerge during advanced stage of tumors in circulation. These can be detected in peripheral blood and other biofluids, providing minimally invasive tools for diagnosis, prognosis, and therapeutic monitoring. [15]

Circulating Tumor Cells (CTCs) as Cancer Biomarkers

CTCs are malignant cells that detach from primary or metastatic tumors and enter the bloodstream. Their presence reflects tumor invasiveness and metastatic potential, making them valuable biomarkers for cancer diagnosis, prognosis, treatment monitoring, and personalized therapy through liquid biopsies. While conventionally used for monitoring, CTCs are increasingly emerging as tools to aid in early cancer detection and personalized therapies. [15,21]

CTCs act as biomarkers:

- **Early Detection:** CTCs can be detected in the blood prior to conventional imaging techniques so their detection can reveal tumors at earlier stages of cancer. [21]
- **Prognosis:** The presence and quantity of CTCs in peripheral blood are strongly correlated with disease stage and clinical outcomes across multiple cancer types. Elevated CTC counts are frequently associated with advanced disease, increased metastatic burden, and poorer overall survival. In invasive breast cancer, the presence of CTCs has been shown to

determine patient survival at various periods during treatment. Cancer treatment targets (CTTs) have been documented as better predictors of prognosis than conventional tumor markers (e.g., CA27-29). CTC counts have been confirmed to be a reliable prognostic indicator in metastatic prostate cancer. [15, 21]

- **Treatment Monitoring:** CTCs can be useful in therapeutic monitoring. Reduced CTCs post- treatment might be indicative of a positive treatment response, while increased numbers could suggest treatment failure or recurrence. [15, 21]

Clinical applications of CTCs

Various molecular approaches utilized for the diagnosis of CTCs include DNA sequencing, RNA sequencing, RNA in situ hybridization, and chromatin immunoprecipitation sequencing. [15]

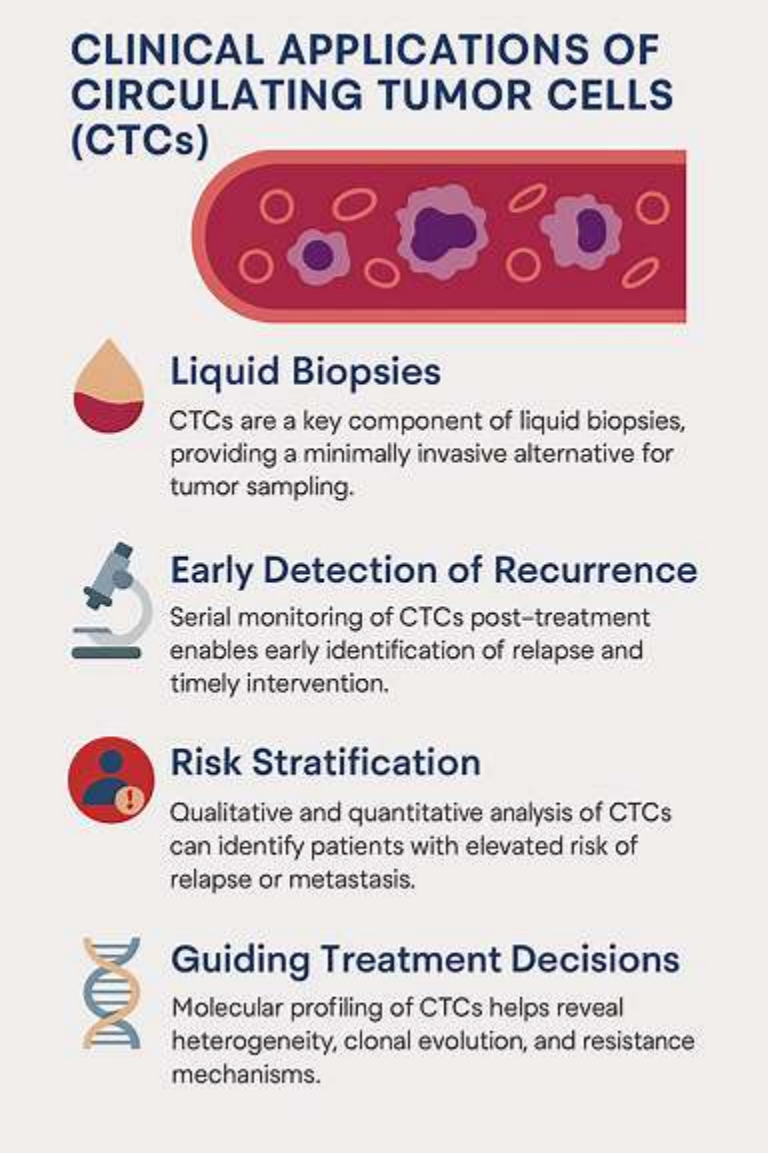


Figure 1: Clinical Applications of CTCs [21]

Cancer Stem Cells (CSCs) as Cancer Biomarkers

Numerous solid cancers, particularly prostate cancer, glioblastoma, breast cancer, medulloblastoma, and melanoma, have been shown to contain CSCs. Cluster of Differentiation (CD) CD24, CD133, CD166 (ALCAM), CD44, CD29, epithelial cell adhesion molecule (EpCAM), leucine-rich repeat-containing G protein-coupled receptor 5(Lgr5), aldehyde dehydrogenase 1 family member B1 (ALDH1B1), and aldehyde dehydrogenase 1 family member A1 (ALDH1A1) are some of the cytoplasmic and surface markers which have been utilized to detect putative cancer CSCs. [15]

Immune Cells as Cancer Biomarkers

Immune cells can serve as potential cancer biomarkers, providing insights into disease prognosis, therapeutic response, and even early detection. Precisely, the presence, type, and quantity of immune cells within a tumor can indicate the nature of treatment response to immunotherapies like checkpoint inhibitors. [15]

Tumor-infiltrating lymphocytes (TILs), macrophages, dendritic cells, neutrophils, circulating immune cells are immune cells biomarkers. [15,22]

- **Tumor mutational burden (TMB):**

TMB indicates the number of genetic mutations in a tumor. Tumors with high TMB show more probability to express abnormal proteins (neoantigens) that can be targeted by the immune system, and are often associated with better responses to immunotherapy. [15,22]

- **Programmed death-ligand 1 (PD-L1) expression:**

The expression of protein PD-L1 on tumor cells and immune cells act as a biomarker for predicting response to checkpoint inhibitor therapies targeting the PD-1/PD-L1 pathway. [15,22]

Clinical studies have reported that lung adenocarcinoma (LUAD), melanoma (MEL), lung squamous carcinoma (LUSC), and head and neck squamous carcinoma (HNSC) all had a high incidence of CD8-hot, CD3-hot, and PD1-hot tumors. [15,22]

Other Biomarkers [15]

Other categories of cancer biomarkers include: i) lamins(A, B, C, A/C, & B1), ii) galectins (Galectin 1,2,3,4,5,6,7,8,9 & Galectin 12), iii) carbohydrate antigen (CA), iv) virus-induced hepatocellular carcinoma (HCC) and human papillomavirus (HPV) markers, v) exosomes specific biomarkers (specific microRNAs (miRNAs), proteins like EGFR, CD91, and P-glycoprotein, long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs)), vi) lipid based biomarkers (phosphoinositide, cholesterol, prostaglandin, lysophosphatidic acid, sphingosine 1 phosphate & ceramides). [15]

Classification of Cancer Biomarkers

Diagnostic (screening) Biomarkers

Diagnostic and screening biomarkers play a pivotal role in **early cancer detection**, enabling timely clinical interventions and improving patient outcomes. These biomarkers are typically measurable substances or molecular signatures found in blood, tissue, or other bodily fluids. Figure 2 displays some of the examples for early detection of different cancers. [15]

Biomarker	Cancer Type	Sample Type	Clinical Utility
PSA (Prostate-Specific Antigen	Prostate Cancer	Blood (serum)	Screening and monitoring disease progression
FOBT (Fecal Occult Blood Test)	Colorectal Cancer	Stool	Non-invasive early detection of occult bleeding
CEA (Carcinoembryonic Antigen (CA125)	Colorectal, Lung, and Other Cancers	Blood (serum/ plasma)	Detection prognosis, and recurrence monitoring
HPV Testing	Cervical Cancer	Cervical swab or liquid-based cytology for screening & triage	

Fig.2: Diagnostic Cancer Biomarkers for Early Detection [15]

Prognostic Biomarkers

Prognostic biomarkers help predict the likely clinical outcome of cancer, including disease progression, survival probability, and therapeutic response. These markers guide therapeutic decisions and risk stratification across cancer types (Figure 3). [15,26]

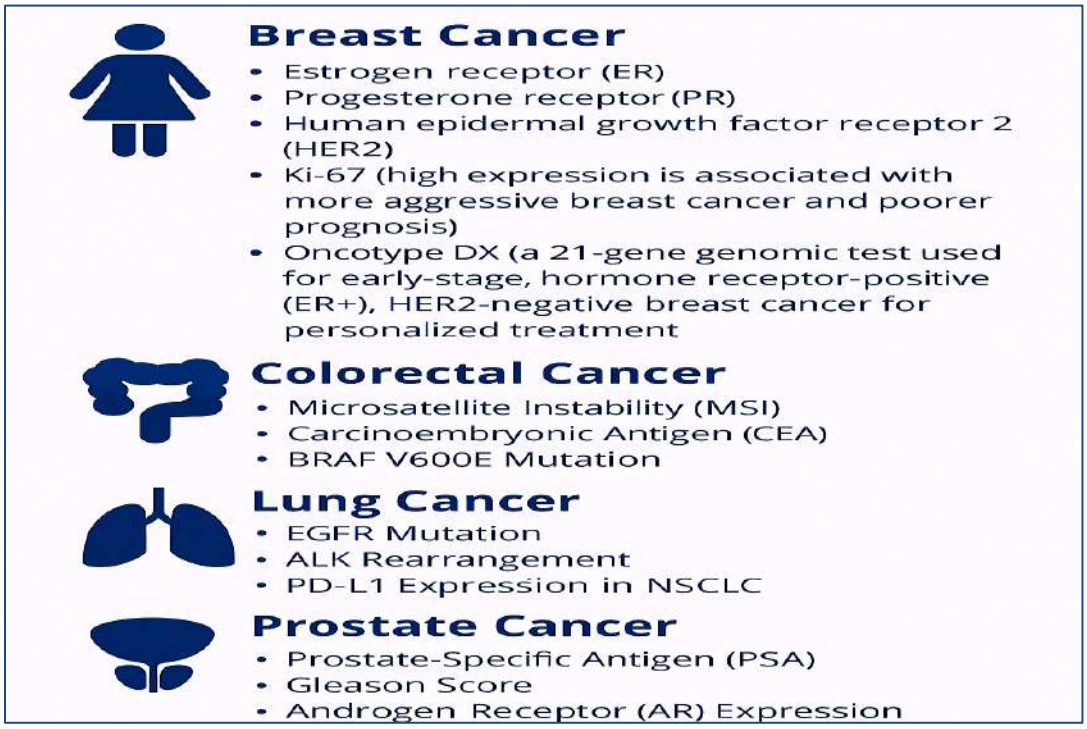


Fig.3: Prognostic Biomarkers in Cancer [15, 26]

Predictive (stratification) Biomarkers

Predictive biomarkers in cancer are used for identification of favourable and unfavourable response to targeted therapies. They guide treatment selection and help personalize therapeutic strategies (Table 3). [15]

Table 3: Predictive Biomarkers and Targeted Therapies [15, 23-25]

Biomarker	Cancer Type	Therapeutic Implication	Targeted Therapies
HER2 (Human Epidermal Growth Factor Receptor 2)	Breast Cancer	Predicts response to HER2-targeted monoclonal antibodies	Trastuzumab, Pertuzumab
EGFR (Epidermal Growth Factor Receptor) mutations (Exon 19 deletions, L858R)	Non-Small Cell Lung Cancer (NSCLC)	Predicts response to EGFR TKIs	Gefitinib, Erlotinib, Osimertinib (Tyrosine Kinase Inhibitors)
ALK (Anaplastic Lymphoma Kinase) rearrangements	NSCLC	Predicts response to ALK inhibitors	Crizotinib, Alectinib, Brigatinib
MSI-H (Microsatellite Instability-High) / dMMR (Deficient Mismatch Repair)	Colorectal Cancer	Predicts response to immune checkpoint inhibitors	Pembrolizumab, Nivolumab
BRAF V600E (B-Raf Proto-Oncogene, Serine/Threonine Kinase) mutation	Melanoma	Predicts response to BRAF and MEK inhibitors	Vemurafenib, Dabrafenib (BRAF inhibitors), Trametinib, Cobimetinib (MEK inhibitors)
ERCC1 (Excision Repair Cross-Complementation Group 1) expression	NSCLC	Explored as predictor for platinum-based chemotherapy efficacy	Cisplatin, Carboplatin

Table 4: Molecular Diagnostics in Clinical Oncology [1]

Category	Analyses	Targets / Purpose	Target Group
Hereditary Cancer Syndromes	PCR (recurrent mutations), Sanger, multiplex ligation-dependent probe amplification (MLPA), NGS panels, Whole genome analysis	**Cancer patients** Risk of second malignancy, Therapy selection **Healthy people** Risk-assessment	Cancer patients, Healthy people
Predictive Markers	DNA, RNA, Proteins, Tissue slices, Patient-derived xenografts (PDxs)	HER2, EGFR-MUT, ALK, BRAF-MUT,	Cancer patients

		ROS, MSI-H, BRCA-mess, chemosensitivity	
Circulating Tumor Fragments	CTCs, ctDNA, RNA, Proteins	**Cancer patients** Tumor burden monitoring, Therapy guidance Tumor eradication control **Healthy people** EarlyDiagnosis	Cancer patients, Healthy people
Carcinomas of Unknown Primary	Single markers, Integrative markers	Tissue-specific (RNA, proteins), Tumor-specific (mutations, CNVs)	Cancer patients

The utilization of molecular-based assays is increasingly becoming an invaluable means in numerous major areas in cancer medicine. Routine molecular testing for hereditary cancer syndromes both for risk-assessment and personalized systemic treatment is now becoming essential in any state-of-the-art laboratory. There are numerous predictive tests involving either the analysis of individual drug targets or identification of specific tumor phenotypes, which guide in the personalized therapeutic regimen for a patient. Molecular detection of residual tumor fragments can aid in monitoring of malignancy; and liquid biopsy is emerging as an important tool for early detection and screening of cancer in the future. The cancers of unknown primary site can be diagnosed by using novel techniques involving the mutation testing and RNA analysis. [1]

Molecular Techniques for Cancer Diagnosis: Advantages and Pitfalls

Recently, the integration of molecular techniques has substantially transformed the understanding of tumor dynamics, treatment response, and resistance mechanisms. Molecular techniques such as PCR, Maxam-Gilbert sequencing, Sanger sequencing, Next Generation Sequencing (NGS), Single-Molecule Real-Time Technology (SMRT), Western Blotting, Fluorescence in situ hybridization (FISH), Comparative Genomic Hybridization (CGH), Single Nucleotide Polymorphism Array (SNP), proteomic techniques, Immunohistochemistry (IHC), Chromatin Immunoprecipitation (ChIP), and Circulating Tumour DNA Analysis (ctDNA) provide exceptional levels of sensitivity, precision, and adaptability in determining genetic and proteomic alterations in cancer. [27]

Core Molecular Techniques

Polymerase Chain Reaction (PCR): PCR-based technologies have become indispensable in molecular oncology, offering exceptional sensitivity for detecting genetic alterations at low mutant allele frequencies (MAFs ≤ 0.1%). The three principal PCR subtypes—digital PCR (dPCR), real-time quantitative PCR (qPCR), and reverse transcriptase PCR (RT-PCR)—enable precise detection of mutations, gene amplifications, and circulating tumor DNA (ctDNA). [28]

- **Real-Time Quantitative PCR (qPCR):** Designed to detect tumor-specific mutations in **cell-free DNA (cfDNA)**. It enhances sensitivity by selectively amplifying mutant alleles [28]
- **Key qPCR-based methods**:
  - **Amplification-refractory mutation system PCR(ARMS-PCR):** Utilizes

allele-specific primers to detect point mutations with high specificity. [28]

- **Peptide-nucleic-acid-locked nucleic acid (PNA-LNA) Clamp PCR**: Employs peptide nucleic acids to suppress wild-type DNA amplification, enriching mutant alleles. Demonstrated 72.7% sensitivity and 100% specificity for EGFR mutations in advanced NSCLC; 0% sensitivity and 100% specificity in early-stage NSCLC. [28]
- **Co-amplification at lower denaturation temperature PCR (COLD-PCR)**: A single-step method that enriches both wild-type and low-frequency mutant alleles by exploiting differential denaturation temperatures. [28]

➢ **Droplet Digital PCR (ddPCR)**

- Exhibits **high precision**, **low copy variant detection**, and **robust inhibitor tolerance** (Figure 4). [28]
- Facilitates a calibration-free quantification signifying a superior advantage over qPCR. [28]
- In a study of 29 preliminary breast cancer patients, ptDNA (plasma Tumor DNA)/ddPCR detected PIK3CA mutations in plasma tumor DNA (ptDNA) with 93.3% sensitivity and 100% specificity. [27]
- Particularly valuable for **liquid biopsy**, **MRD monitoring**, and **mutation tracking** in solid tumors. [28]

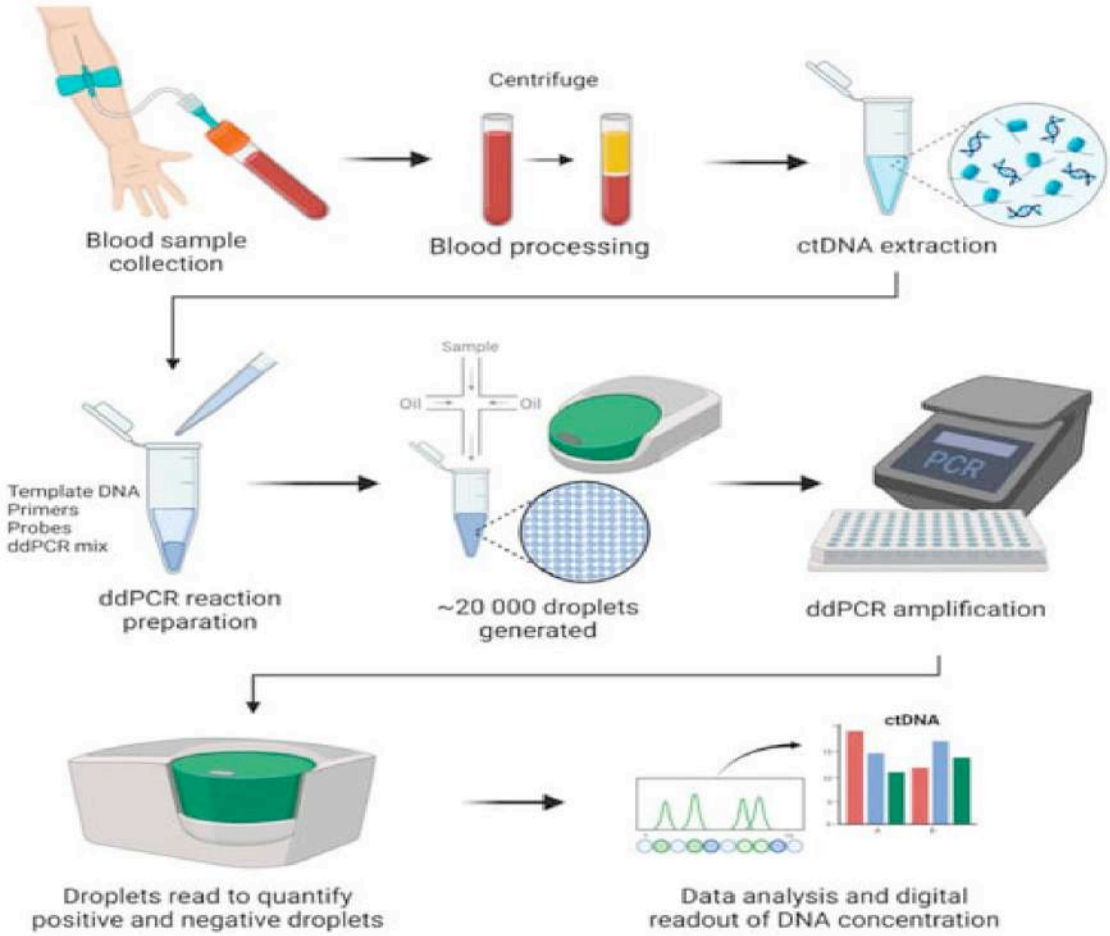


Fig. 4: Stepwise representation of ddPCR in cancer liquid biopsies (Adapted from Gezer et al.) [28]

Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) allows wide-ranging genomic profiling by sequencing 150–400 base pair DNA fragments in massive parallel, facilitating for the identification of driver mutations and guiding personalized cancer therapies. Unlike traditional gel or capillary electrophoresis, NGS platforms use clonal amplification and real-time sequencing during DNA polymerase activity to generate complementary strands. One such platform, Ion Torrent by Thermo Fisher Scientific, employs semiconductor-based detection and requires as little as 1 ng of DNA input, making it suitable for limited or degraded samples. It can detect a wide range of genomic alterations including single-nucleotide polymorphisms (SNPs), insertions and deletions (indels), copy number variations (CNVs), and gene fusions, thereby supporting precision oncology with high sensitivity and efficiency. [27] NGS is a high-throughput, parallel-sequencing technology that enables the detection of genomic variants at both DNA and RNA levels. The clinical NGS workflow involves several key steps: extraction of nucleic acids from tumor cells, target enrichment, library preparation, massive parallel sequencing, bioinformatics analysis, and interpretation of variants. Recently, targeted sequencing is gaining interest current clinical NGS applications focus on the analysis of targeted gene panels that include actionable targets in oncological diseases. Targeted sequencing uses either amplicon-based or hybridization capture methods to enrich regions of interest, allowing for the detection of single nucleotide variants (SNVs), small insertions or deletions (indels), copy number variants (CNVs), gene fusions, and other structural alterations. Whole-genome (WGS) and whole-exome sequencing (WES)approaches also enable the identification of complex biomarkers such as tumor mutational burden and mutational signatures linked to DNA repair deficiencies (e.g. mismatch repair, homologous recombination) and tumor etiologies. [29] However, implementing NGS in resource-limited settings requires careful consideration of infrastructure, cost, and accessibility. [27]

Next-generation sequencing (NGS) and polymerase chain reaction (PCR) are both widely used diagnostic tools, but NGS offers greater precision in detecting genomic alterations. Unlike real-time PCR, which may miss certain variants—such as specific insertion-deletions and nonsynonymous single-nucleotide changes—NGS can reliably identify these mutations. The throughput and scope of PCR-based approaches are limited; they are only able to search for known variations and are sensitive and affordable. NGS can screen for both known and novel genomic modifications, providing a more comprehensive view of the mutational landscape. [30]

BOX 1: Recommendations from European Society for Medical Oncology (ESMO 2024) for the use of NGS in patients with advanced cancer: [31]

General Principles

- Multigene NGS is recommended for patients with advanced cancers where tumour-agnostic therapies are accessible.
- Cost-effectiveness should be evaluated locally before implementation.
- Use of ESCAT (ESMO Scale for Clinical Actionability of molecular Targets) is encouraged to prioritize clinically relevant targets.

Lung Cancer

- Tumour NGS is recommended in advanced non-squamous NSCLC as part of routine practice.

Breast Cancer

- For HR-positive/HER2-negative advanced breast cancer (ABC):
  - NGS should be performed after endocrine therapy resistance to detect ESR1 mutations.
  - Germline BRCA1/2 testing is advised for high-risk patients, even if tumour NGS is negative.

Colorectal Cancer (CRC)

- Multigene tumour NGS is recommended if it does not incur additional cost compared to standard methods (IHC, PCR, Sanger).

Prostate Cancer

- NGS is recommended in settings where PARP inhibitors (PARPi) are available.

Ovarian Cancer

- NGS should be combined with HRD (homologous recombination deficiency) signature testing in advanced high-grade ovarian cancer.

Rare and Other Tumours

- Advanced cholangiocarcinoma (CCA)
- Advanced gastrointestinal stromal tumour (GIST)
- Advanced soft tissue sarcomas
- Advanced thyroid cancer

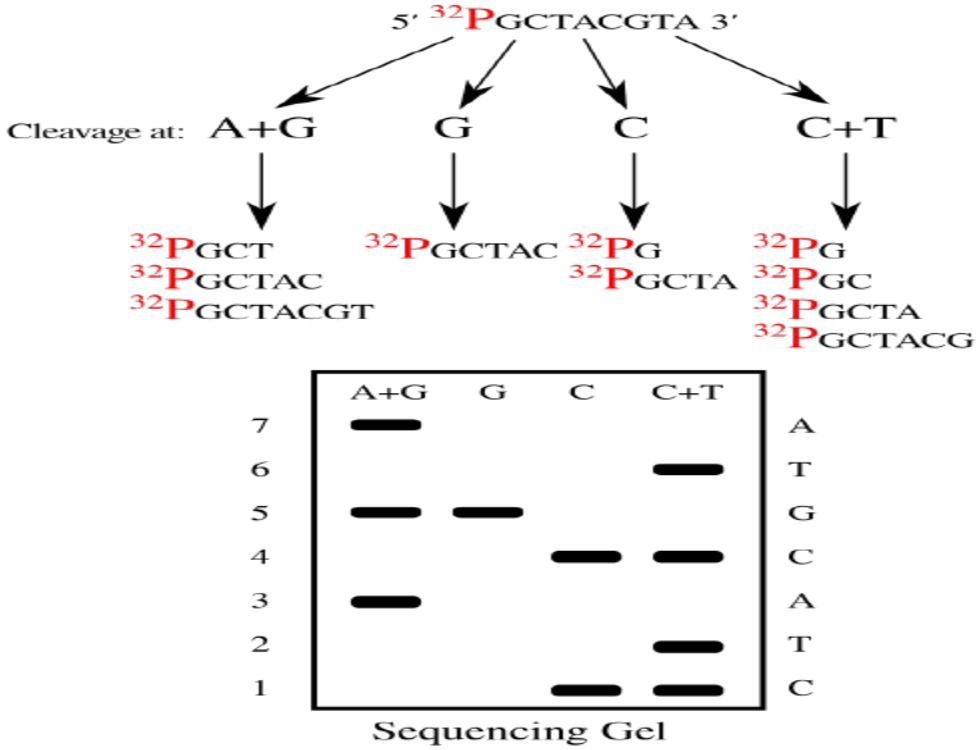


Fig 5: Maxam-Gilbert DNA sequencing method (Source: File:Maxam-Gilbert sequencing en.svg - Wikimedia Commons)

In Sanger sequencing, DNA synthesis occurs by using radiolabelled 2′,3′-dideoxynucleoside (ddNTP) as the source of modified nucleotide triphosphate. The ddNTP(dideoxy), lacks the 3′-hydroxyl group required for the formation of phosphodiester bond with an incoming regular nucleotide (i.e., deoxynucleoside triphosphate, or dNTP), therefore it terminates the chain extension, creating fragments of different lengths. These fragments are then separated by size, typically through capillary electrophoresis, and a laser detects the fluorescent signal of the last ddNTP, which reveals the DNA sequence. Sanger sequencing is considered as "gold standard" method for sequencing and it is relatively easier than Maxam-Gilbert method of sequencing The most significant SNVs and minor insertions/deletions are detected by Sanger sequencing (Figure 6). [27, 32]

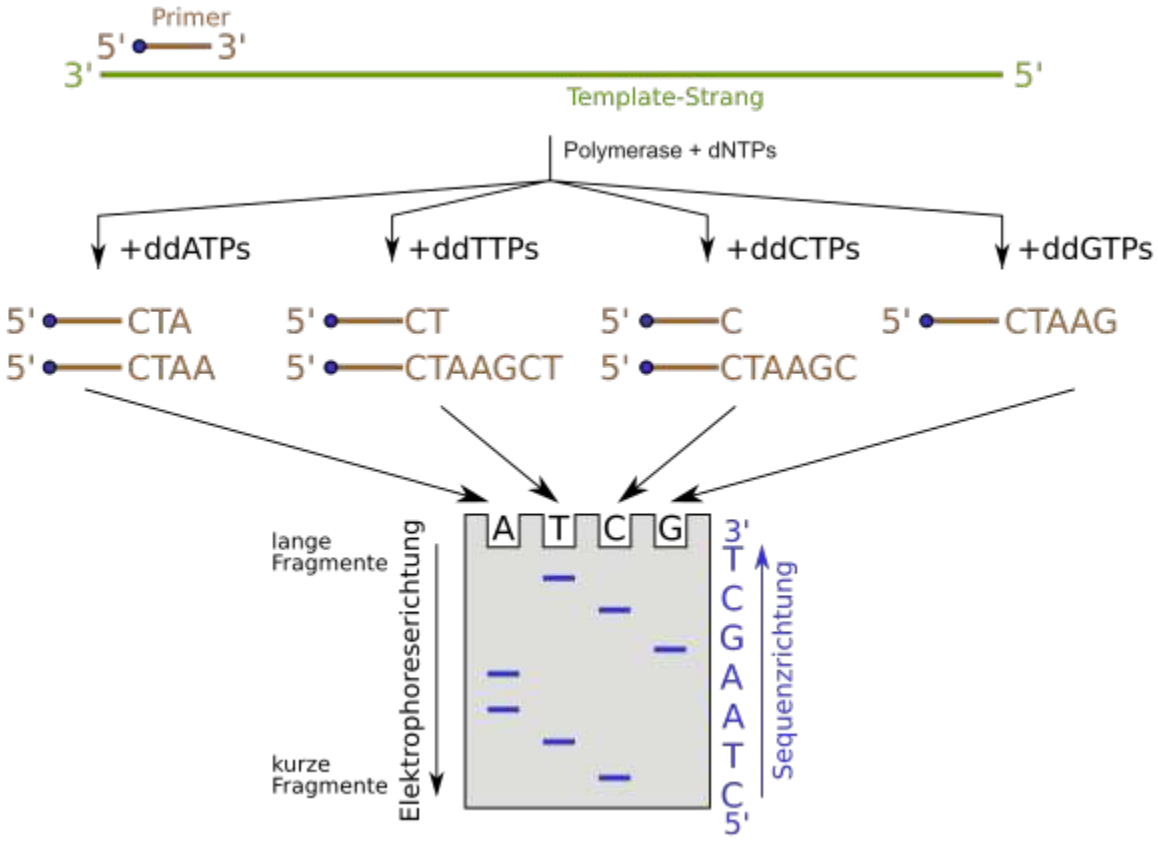


Fig. 6: Sanger sequencing method
(Source: https://commons.wikimedia.org/wiki/File:Didesoxy-Methode.svg)

Single-Molecule Real-Time (SMRT) & Nanopore Sequencing:

Long-read sequencing technologies such as SMRT and nanopore sequencing offer powerful tools for analyzing complex mutations. In SMRT sequencing by Pacific Biosciences, DNA synthesis is monitored in real time as a polymerase incorporates fluorescently labeled nucleotides within a nanostructure called a zero-mode waveguide (ZMW). The ZMW confines illumination to a tiny detection volume, allowing precise observation of each base addition. In contrast, nanopore sequencing by Oxford Nanopore Technologies detects individual nucleotides by measuring changes in ionic current (ion flow across the pore) as single-stranded DNA passes through a nanopore embedded in a membrane. These approaches enable long-read capabilities essential for resolving structural variants, repetitive regions, and intricate genomic rearrangements. (Figure7). [27, 32]

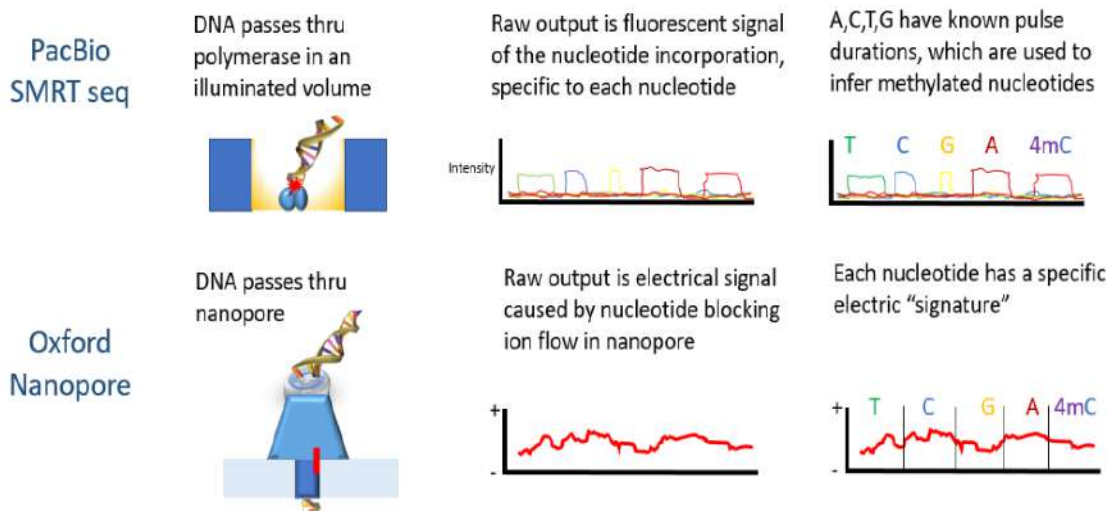


Fig. 7: (A) The technology of SMRT sequencing and (B) the technology of nanopore sequencing (Source: File:3rd gen Epigenetics.png - Wikimedia Commons)

Automated Sequencing Method

The development of automated DNA sequencing significantly advanced the use of Sanger sequencing by replacing radiolabeled nucleotides with fluorescently labeled ones. Each of the four dideoxynucleotides (ddA, ddT, ddC, ddG) is tagged with a distinct fluorescent dye, allowing all chain-termination reactions to occur in a single reaction tube. During capillary electrophoresis, DNA fragments are separated and detected based on the unique emission spectra of these dyes. This innovation not only eliminated the need for radioactive reagents but also enabled faster, safer, and more streamlined automated sequencing workflows, paving the way for high-throughput automation. (Figure 8). [27,32]

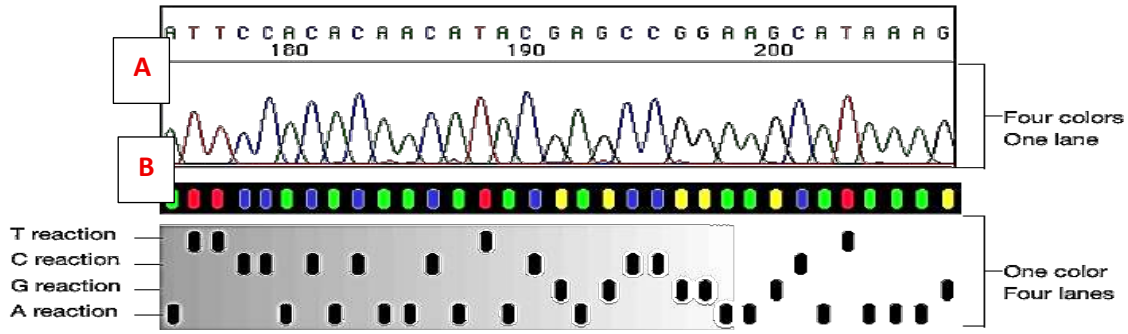


Fig. 8: Difference between fluorescent sequencing (A-automated capillary electrophoresis) versus radioactive sequencing (B-conventional gel electrophoresis) (adapted from DNA Sequencing by Capillary Electrophoresis Applied Biosystems. Applied Biosystems Chemistry Guide. 2nd Edition [33]

Genome Editing

Genome editing is a powerful technique that enables precise modifications to an organism's DNA at specific genomic sites. Among the most transformative advancements in this field is the CRISPR/Cas9 system, introduced in 2013, which has rapidly become the cornerstone of targeted genome editing due to

its efficiency and versatility. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and its associated enzyme CRISPR-associated nuclease 9 (Cas9) allow researchers to perform gene knock-outs, knock-ins, repairs, and even transcriptional regulation with high specificity. This technology has revolutionized genetic research and therapeutic development, offering unprecedented control over gene function and expression. [30]

The CRISPR/Cas9 system comprises three key components: the Cas9 endonuclease, CRISPR RNA (crRNA), and transactivating crRNA (tracrRNA). For genome editing applications, this system is streamlined by fusing crRNA and tracrRNA into a single-guide RNA (sgRNA), The CRISPR/Cas9 system was synthesized by fusing single-guide RNA (sgRNA) and Cas9. Upon binding, the Cas9 protein induces a double-strand break near the protospacer adjacent motif (PAM). Specifically, the RuvC domain cleaves the non-complementary DNA strand, while the His-Asn-His (HNH) domain targets the strand complementary to the sgRNA. (Figure 9 A& B). [30]

Among the most compelling uses of CRISPR-Cas9 in cancer immunotherapy is the development of universal Chimeric Antigen Receptor (CAR)-T cells. [30]

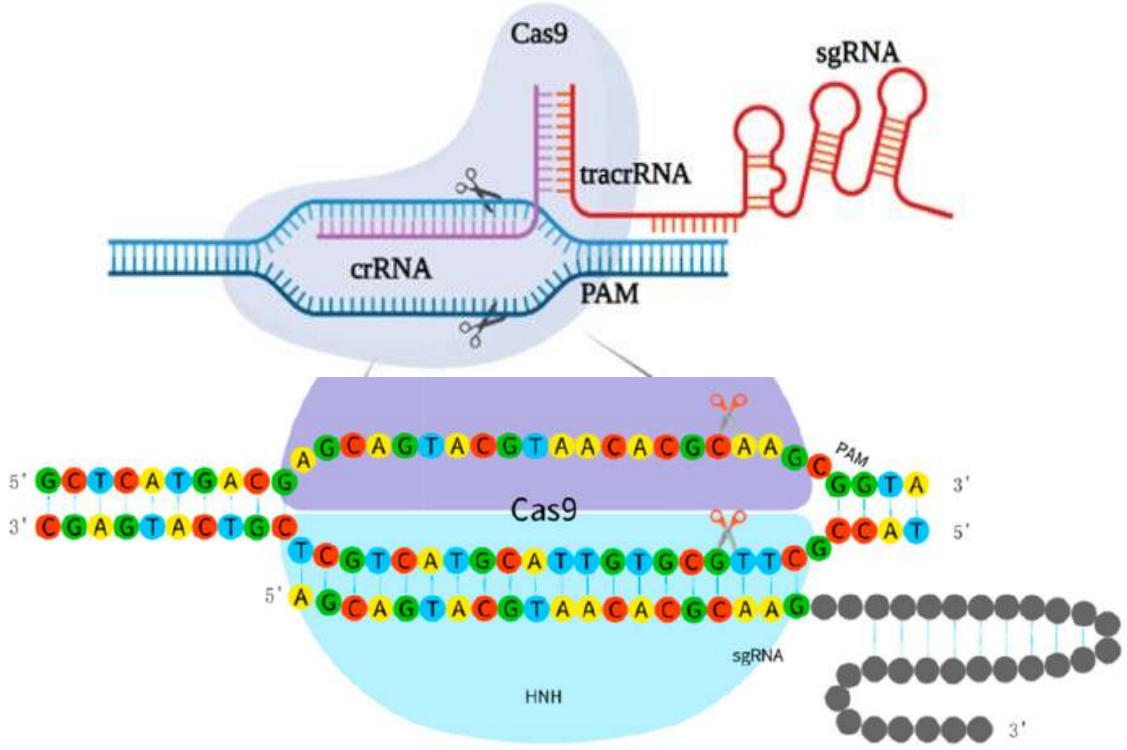


Fig. 9(A): Mechanism of CRISPR/Cas9 (Adapted from Selvakumar etal; 2022 [30]; Source: File:The schematic diagram of CRISPR-Cas9.webp - Wikimedia Commons)

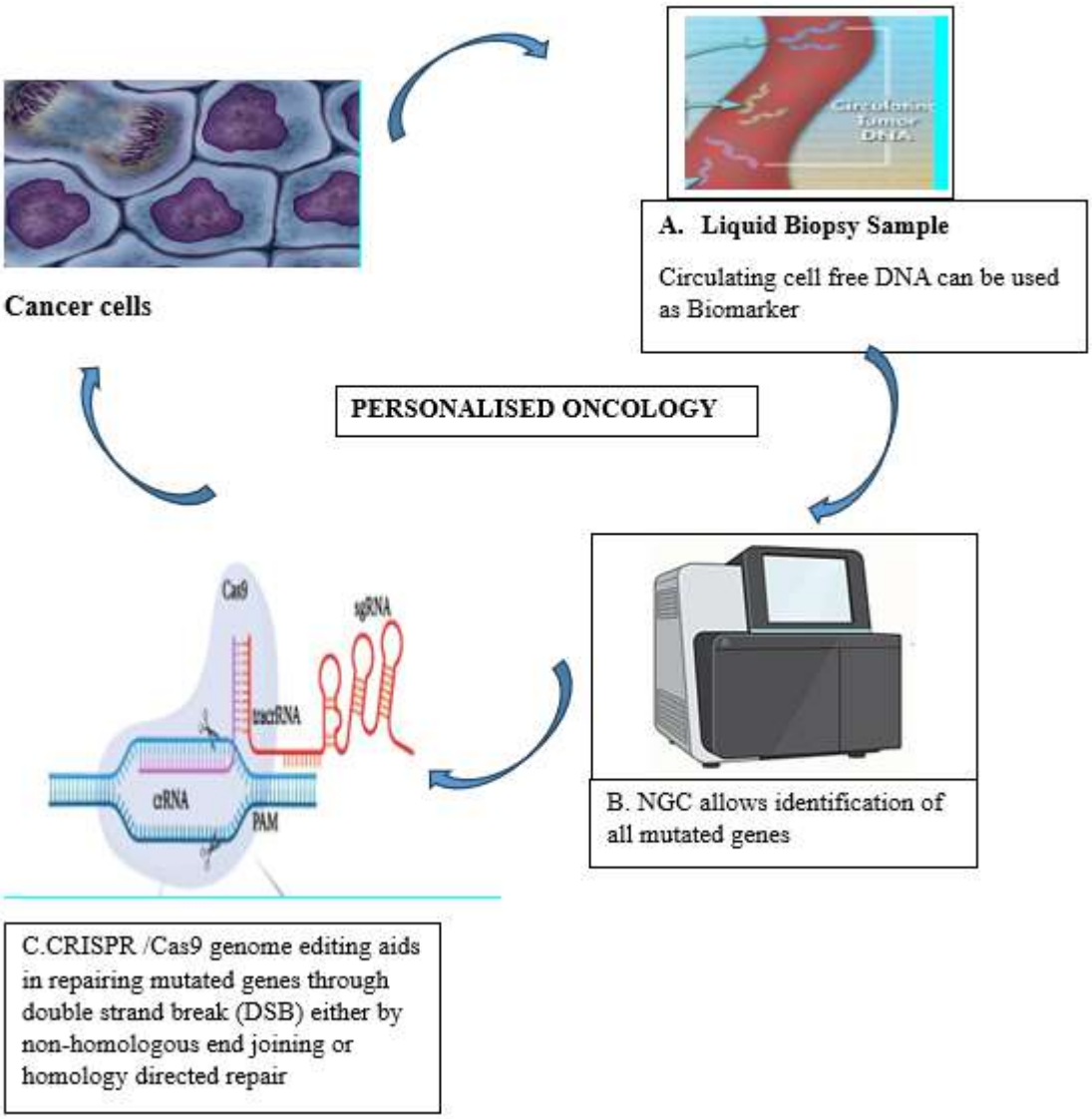


Fig. 9 (B): Application of Next Generation Sequencing (NGS) and CRISPR/Cas9 in personalised oncology (Adapted from Selvakumar etal; 2022 [30])

Complementary Diagnostic Tools

Western Blotting (WB) Technology

Western blotting is a highly sensitive and specific technique widely used in medical research to detect and quantify proteins, especially those present in low abundance within diagnostic samples. It plays a supportive role in diagnosing conditions like Ewing sarcoma. [27] Programmed cell death 1 (PD-1) (PD-1) and programmed cell death ligand 1 (PD-L1 (PD-L1) are critical immune checkpoint proteins that help maintain immune homeostasis by preventing autoimmunity during immune responses. Along with cytotoxic T-lymphocyte-associated protein (CTLA-4), these inhibitory molecules can suppress antitumor T-cell activity. Many solid tumors—including non-small cell lung carcinoma, malignant melanoma, and urothelial carcinoma—exploit this mechanism by upregulating PD-L1 expression, thereby deactivating cytotoxic T lymphocytes (CTLs) and evading immune surveillance. Western

blotting, a sensitive and specific protein detection method, is frequently used alongside immunohistochemistry (IHC) to confirm PD-L1 expression, supporting both diagnostic accuracy and therapeutic decision-making in oncology. [34] WB technology enables specific identification of proteins at the single-cell level. An improved single -cell WB (ScWB) approach is used to investigate stem cell signaling and differentiation, and response to drugs in the tumor cells. ScWB approach has been applied to analyze oncoprotein-related signaling in human breast biopsy samples, demonstrating its clinical utility (Figure 10). Moreover, WB plays a crucial role in investigating multidrug resistance (MDR) by assessing the expression of MDR-1/P-glycoprotein in resistant and non-resistant cell lines such as P388/ADR (adriamycin), P388, and human colon tumor-cell line 15(HCT-15), aiding in the understanding of resistance mechanisms and therapeutic targeting. [35]

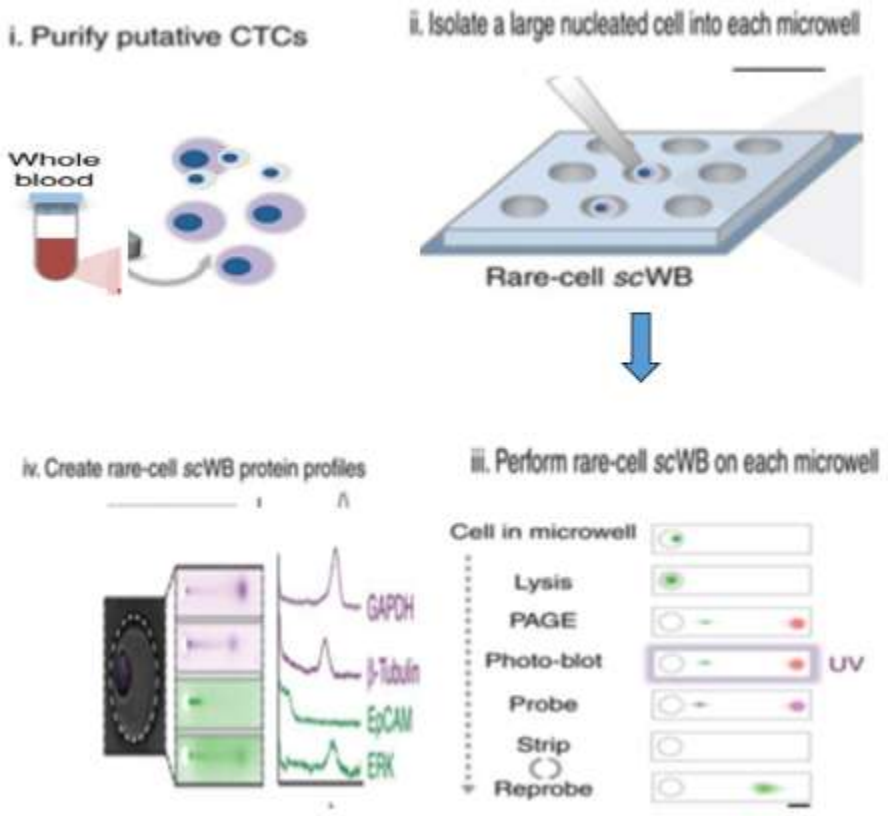


Fig.10: Profiling protein expression using microfluidic WB in circulating tumour cells (Adapted from Sinkala etal). [36]

Fluorescence In Situ Hybridization (FISH)

Fluorescence in situ hybridization (FISH) is a molecular cytogenetic technique that uses fluorescently labeled DNA probes to detect and localize specific DNA sequences on chromosomes within metaphase spreads, interphase nuclei, and paraffin-embedded tissue sections (in situ). It enables the identification of chromosomal aberrations such as aneuploidies, duplications, deletions, inversions, and translocations—whether congenital or acquired. (Figure 11). [27]

Main applications of FISH in clinical oncology:

- Detection of gene amplification (e.g., human epidermal growth factor receptor 2(HER2) in breast / gastric / colorectal / endometrial cancer) [37]
- Detection of gene or chromosomal deletion (e.g., tumor protein p53(TP53) deletion on chromosome 17p in myeloid neoplasms) [37]
- Detection of chromosomal translocation (e.g., anaplastic lymphoma kinase (ALK) translocation on lung cancer) [37]
- Detection of gene translocation and amplification (e.g., MYC oncogene in Burkitt's lymphoma; 90% cases have translocation of c-myc or variants) [38]
- Detection of gene translocation (e.g., cyclin D1 in mantle cell lymphoma) [39]
- Detection of gene translocation (e.g., promyelocytic leukemia protein:: retinoic acid receptor alpha (PML:RARA; PML::RARA is found in 98% of acute promyelocytic leukemia (APL) cases and has not been documented in other neoplasms) [40]
- Detection of aneuploidy (in the 9p21 region, containing the p16/CDKN2A gene in bladder cancer) [37]

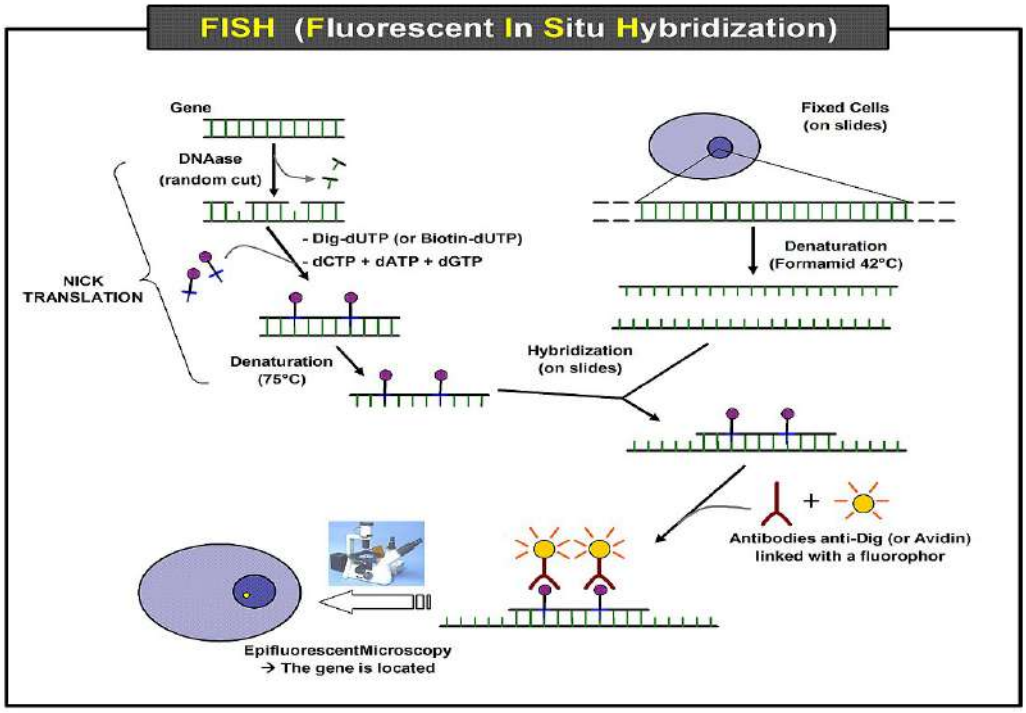


Fig.11: Schematic representation of fluorescent in situ hybridization (FISH) (Source: Fluorescence in situ hybridization - Wikipedia)

Comparative Genomic Hybridization (CGH)

Comparative Genomic Hybridization (CGH) is a molecular cytogenetic technique that enables genome-wide detection of copy number variations (CNVs), identifying chromosomal gains and losses without prior knowledge of target regions. It is increasingly applied in cancer genetics to uncover genomic alterations linked to tumor initiation, progression, and metastasis. This technique offers examination of entire genome in a single reaction therefore there is no need to know the genetic region of interest before investigation. Once genomic regions showing copy number gain or loss are identified—typically through array CGH

(aCGH)—these areas can be further refined using FISH, which provides spatial resolution at the cellular level. aCGH involves co-hybridizing differentially labeled tumor and normal DNA samples onto a microarray composed of immobilized DNA probes arranged on a solid surface, such as a glass slide in an ordered manner. In aCGH, the relative copy number at each locus is estimated by measuring the fluorescence intensity ratio of tumor DNA to normal DNA hybridized to specific probes on the array. This ratio reflects the degree of amplification or deletion at that genomic site, enabling precise mapping of chromosomal aberrations. [27] An association of DNA copy number aberrations with prognosis has been found for a variety of tumor types, including lymphomas, chronic lymphocytic leukemia, gastric cancer, prostate cancer, and breast cancer. For example: In breast cancer, extensive studies have shown that specific copy number aberrations (CNAs) correlate with key clinicopathologic features such as tumor grade, estrogen receptor (ER) status, TP53 mutation, molecular subtype, and overall survival outcomes. Detection of these CNAs might provide insights on the genes crucial in contributing to breast cancer development and progression, and might aid in improved prognosis with breast cancer (Figure 12). [41]

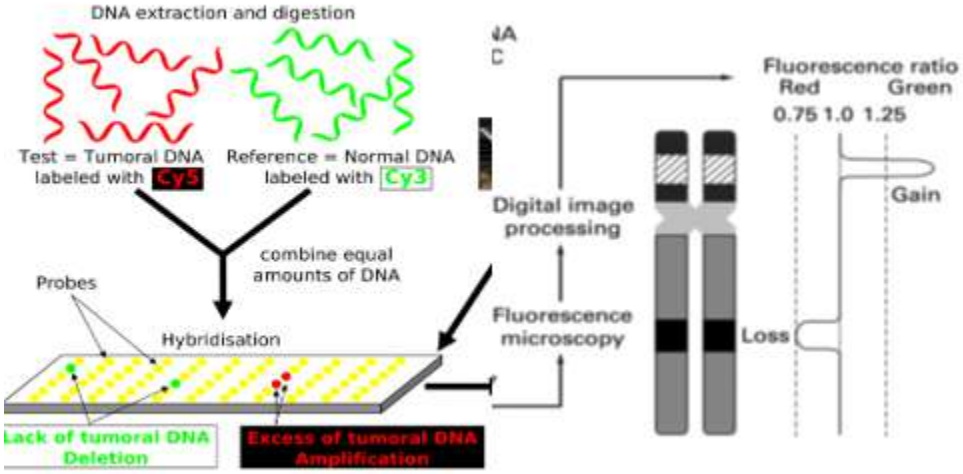


Fig.12: aCGH stepwise procedure (Adapted from Budhbaware etal (2024) [27]; Source: File:Array-CGH protocol.svg - Wikimedia Commons)

Single Nucleotide Polymorphism Arrays (SNPs)

SNP arrays and SNP-seq are powerful tools for characterizing genetic variation and tumor biology in cancer. SNP arrays enable genome-wide detection of germline and somatic mutations, copy number alterations, and loss of heterozygosity, including somatic uniparental disomy (UPD), which is particularly relevant in cancer diagnostics. They are increasingly used in clinical workups for melanocytic malignancies. SNP array genotyping can recognize alterations associated with DNA methylation, genetic copy number, and heterozygosity loss in cancer cells. The have revealed the origins of ovarian teratomas and spatial tumor-immune relationships in liver cancer (the spatiotemporal relationships between immune cells and malignancy). Three important steps comprise SNP-seq: i) nuclear protein binding of SNP-containing oligos; ii) separating protein-bound from protein-free oligos; iii) preparing and analyzing sequencing libraries. [27]

Proteomics

Proteomics has become a cornerstone of molecular oncology, offering deep insights into protein expression, modifications, and interactions that drive tumor behavior. Technologies such as mass spectrometry, laser capture microdissection, and 2-D gel electrophoresis enable precise profiling of cancer tissues, facilitating the identification of biomarkers and therapeutic targets. These approaches have unraveled mechanisms underlying tumor growth, metastasis, and drug resistance—critical challenges in cancer treatment.By mapping protein alterations and signaling pathways, proteomics empowers clinicians to make informed decisions, personalize therapies, and improve patient outcomes through targeted interventions. (Table 5). [42]

Table 5: List of different cancer biomarkers identified by proteomic approaches [42]

Cancer biomarkers identified using proteomics approaches			**Cancer biomarkers identified by proteomics approaches immunotherapy**		**Molecular targets in brain cancer identified with proteomics approaches**		
Type of Cancer	**Target**	**Biomarker/ Target Type**	**Type of Cancer**	**Biomarker of Immunotherapy**	**Type of Cancer**	**Target**	**Biomarker/ Target Type**
Liver (HCC)	PYCR2, ADH1A	Prognostic	Liver	SLC10A1	Glioblastoma	YBX1	Prognostic & Therapeutic
Pancreas	LKB1	Prognostic	Melanoma	MHC	Glioblastoma	CD9, EGFRvIII	Therapeutic
Ovary	MAP2	Prognostic	Lung	LAIR1, TIM3	Glioblastoma	LRG1, CRP, C9, CCT6A	Prognostic
Breast	PYCR1 CD66c, NEDD4	Prognostic Therapeutic	Glioblastoma	FAK	Glioma	SDC1	Diagnostic
Lung	PI3K/MTOR	Therapeutic	Colon	IGF2BP3	Glioma	CDH18	Prognostic
Myeloid Leukemia	IL3RA, CD99	Therapeutic	Clear cell renal cell carcinoma	OXPHOS, PRDX4, BAP1, STAT1	Astrocytoma	109 proteins	Prognostic

			Endometrial carcinoma	CDK12			

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) is a vital tool in cancer diagnostics that enables precise visualization of protein biomarkers within tissue sections, offering insights into antigen localization, morphology, and distribution. It plays a key role in identifying prognostic markers, determining tumor origin in cases of uncertain histogenesis, and predicting therapeutic response. In breast cancer, for example, IHC detects estrogen (ER) and progesterone (PR) receptors to guide hormone therapy decisions, and HER2 overexpression to inform targeted treatment with agents like trastuzumab. Additionally, IHC aids in subtyping cancers such as lymphomas and refining tumor grading, thereby enhancing diagnostic accuracy and personalized treatment planning. [43]

Chromatin Immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) assays are powerful tools for profiling protein–DNA interactions, enabling researchers to investigate transcriptional regulation via histone modifications (epigenetics) or transcription factor binding. The merit of ChIP assays relies on their ability to capture a snapshot of specific protein–DNA interactions in vivo and to quantitate the interactions using quantitative polymerase chain reaction (qPCR). The ChIP assay involves four steps: (1) crosslinking of chromatin-binding proteins to DNA, (2) fragmentation of chromatin, (3) immunoprecipitation of target protein using a high-specificity antibody, and (4) identification and quantification of immunoprecipitated DNA using qPCR or NGS (Figure 13). Notable applications include mapping estrogen receptor-α (ERα) binding sites in MCF7 breast cancer cells to elucidate gene regulation pathways, [45] identifying histone modifications like H3K36me3 linked to tumor gene expression in lung cancer, [46] and profiling SOX11 targets in mantle cell lymphoma to assess their influence on patient survival. [47] Additionally, ChIP methodologies are being adapted for liquid biopsy platforms, allowing detection of tumor-specific gene expression signatures from cell-free DNA. [47]

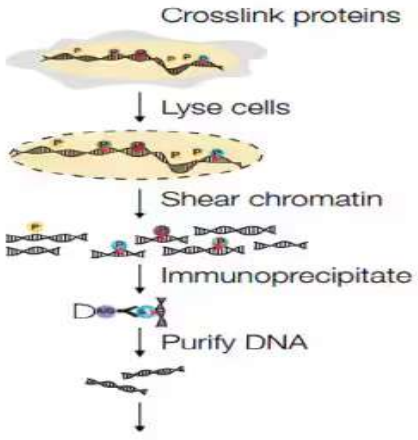
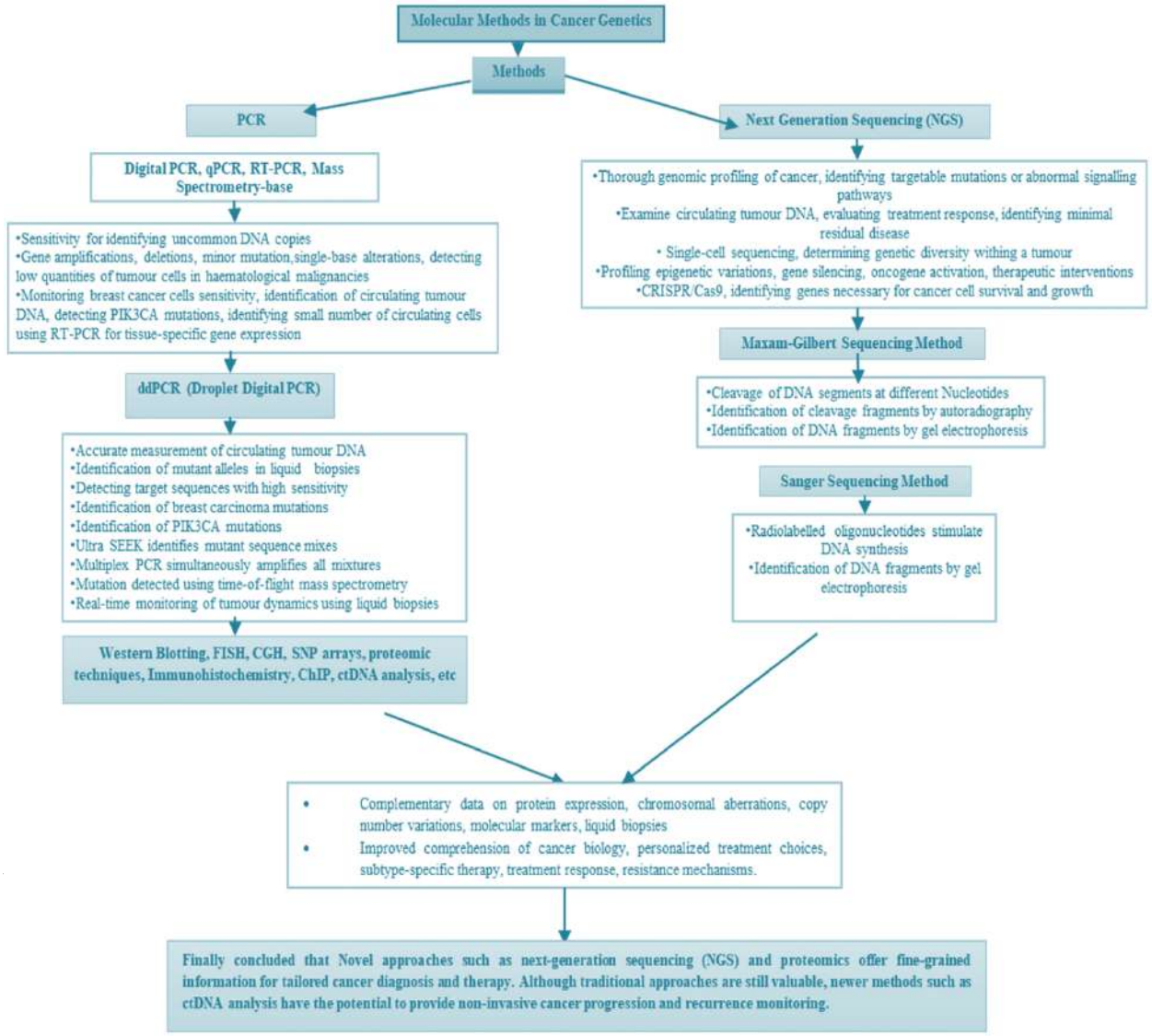


Fig. 13: The chromatin immunoprecipitation (ChIP) assay (Adapted from Thermo Fisher Scientific [44])

Circulating Tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) analysis via liquid biopsy is transforming cancer management by offering a non-invasive method to detect, monitor, and personalize treatment. ctDNA, shed by tumors into the bloodstream, provides real-time insights into tumor genetics, enabling early diagnosis, identification of actionable mutations, and dynamic tracking of treatment response. Technologies like digital PCR and next-generation sequencing (NGS) allow for sensitive detection of minimal residual disease (MRD), which is increasingly used to predict recurrence and guide adjuvant therapy decisions. This approach is gaining traction across cancer types, particularly in lung, breast, and colorectal cancers, and is being integrated into clinical workflows to refine prognosis and therapeutic strategies. [48]



Molecular Diagnostics Roadmap in Oncology
(Adapted from Budhbaware etal; 2024 [27])

Regulation of Molecular Diagnostics

Molecular diagnostics are regulated by the US Food and Drug Administration (USFDA), but the regulations differ depending on whether they are sold as kits or services. [49]

Regulation of Molecular Diagnostic Kits (Medical Devices)

Molecular diagnostic kits for cancer are classified by the USFDA as Class III medical devices due to their potential to significantly influence clinical decisions and patient outcomes. Before these kits can be marketed, they must undergo rigorous validation to ensure analytical accuracy and clinical utility. The FDA offers two regulatory pathways: 1) the "510(k)" route for devices that are "substantially equivalent" to existing devices, which results in FDA "clearance" without requiring safety and efficacy data; 2) the more stringent premarket approval (PMA) route for novel devices, which mandates comprehensive evidence of safety and effectiveness, often through clinical trials. [49]

Molecular Diagnostics Regulated as Services

Molecular diagnostics offered as services are regulated under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, which mandate certification for any laboratory conducting tests on human tissue. CLIA ensures analytical validity—accuracy and reliability of lab processes—but does not evaluate clinical validity or utility. The FDA regulates the essential components used in these tests, such as reagents and instruments, restricting their sale to certified labs, diagnostic manufacturers, or academic institutions. However, for lab-developed tests (LDTs) offered as services, there is currently no formal FDA approval pathway, creating a regulatory gap. Many experts view CLIA accreditation as a relatively low hurdle, raising concerns about oversight and consistency in clinical application. Information on CLIA regulations may be found at the following website: http://wwwn.cdc.gov/clia/. [50]

Impact of Lack of Regulatory Processes in Molecular Diagnostics

The absence of robust regulation in molecular diagnostics poses significant risks to patient care. Without mandatory requirements for clinical validity and utility studies, unproven tests may enter the market, potentially leading to misdiagnoses or inappropriate treatment decisions. This regulatory gap can result in patients receiving suboptimal care or being denied access to beneficial diagnostics due to lack of insurance reimbursement or physician skepticism. Moreover, the lack of standardized oversight contributes to inconsistent test quality, especially in fragmented healthcare systems, and undermines clinician and patient confidence in using molecular diagnostics as reliable tools for precision medicine. [49]

Ethical Challenges with Molecular Diagnostics

Ethical Issues with Molecular Diagnostics for Cancer Risk

Genetic testing for cancer risk raises complex ethical concerns due to the probabilistic nature of most cancer-associated mutations, which often increase risk without guaranteeing disease. Many patients struggle to interpret these risks accurately, leading to unnecessary anxiety or false reassurance. The proliferation of laboratory-developed tests (LDTs) by commercial labs—often including genes with limited or uncertain clinical relevance—further complicates matters, especially when these tests lack robust analytical and clinical validation. [49]

Ethical Issues in Analyzing a Person's Genome

Ethical concerns surrounding the use of tissue samples for both medical (e.g., to develop new molecular diagnostics) are also applicable for and non-medical research (such as ancestry

testing) Individuals must be clearly informed about how their samples and genetic data will be used, stored, and potentially shared, including future applications beyond the original intent. [49]

Ethical, Legal, and Social Implications (ELSI) Program

As molecular biomarkers are evaluated by utilizing molecular diagnostics, ethical issues related to human genome research are also applicable to molecular diagnostics. Many of these ethical considerations were anticipated by the Human Genome Project's program devoted to studying these issues: (Ethical, Legal, and Social Implications-ELSI) research program. The ELSI research program, support both foundational (basic) and practical (applied) studies that explore how genetic and genomic research affects people at multiple levels. More information on ELSI may be found on the website: Ethical, Legal and Social Implications Research Program . [51]

To capture this diversity of issues, the ELSI Research Program has identified four broad areas of research: [51]

- Genomics and Sociocultural Structures and Values
- Genomics at the Institutional and System Level
- Genomic Research Design and Implementation
- Genomic Healthcare

Conclusion

Recent advancements in molecular methods for cancer genetics have significantly expanded the frontiers of precision oncology. AI-integrated next-generation sequencing (NGS) platforms now enable real-time interpretation of complex mutational profiles, improving diagnostic accuracy and therapeutic targeting. Epigenetic biomarkers, particularly DNA methylation signatures, are being incorporated into liquid biopsy assays for early cancer detection. Single-cell multi-omics technologies offer unprecedented resolution of tumor heterogeneity, aiding in resistance monitoring and treatment adaptation. CRISPR-based functional genomics screens are uncovering novel synthetic lethal interactions, while protein interaction mapping is refining drug design by focusing on functional networks. Collectively, these innovations are transforming cancer care by enabling more personalized, dynamic, and effective diagnostic and therapeutic strategies.

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Authors contributions

Sonal Jain was responsible for conceptualization and drafting of this paper

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