The Potential of Cancer Biomarkers

From Discovery to Clinical Application

Edited by
Pranav Kumar Prabhakar

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Preface

The advancement of cancer diagnostics and therapeutics has been significantly shaped by the discovery and development of cancer biomarkers, which have emerged as powerful tools in understanding, diagnosing, and personalizing treatment for cancer patients. *The Potential of Cancer Biomarkers: From Discovery to Clinical Application* brings together cutting-edge research and comprehensive analyses from leading experts to highlight the transformative role biomarkers play across various stages of cancer management.

This book is structured to guide readers through the entire biomarker landscape. The initial chapters delve into the discovery and validation processes, encompassing the molecular mechanisms underlying different biomarkers. Further sections address the methodologies used in biomarker identification and analysis, including recent innovations in genomics, proteomics, and bioinformatics that have propelled the field forward. A distinct focus is placed on translational applications, emphasizing biomarkers in precision medicine, early detection, and as predictive tools in therapy response, ultimately underscoring their clinical impact in improving patient outcomes. This work is intended for a broad spectrum of readers, from researchers and clinicians to graduate students and healthcare professionals, providing a consolidated view of biomarker research and its applications. As cancer treatments become increasingly individualized, the ability to detect and interpret biomarkers accurately will be paramount. We hope that this book will serve as a valuable resource, inspiring new research and practical applications in the realm of cancer biomarkers.

I extend my sincere gratitude to the contributors whose expertise has shaped this book. Their dedication and insights have brought a unique depth to this work, illuminating the complexities and potential of cancer biomarker science. I also appreciate the support of Elsevier in bringing this project to fruition, and I am hopeful that the information and perspectives provided will foster further advancements in cancer care.

Pranav Kumar Prabhakar

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Introduction

The field of cancer research has seen an unprecedented transformation in recent decades, driven largely by the evolution of biomarker science. Biomarkers—biological indicators found in blood, tissues, or other body fluids—serve as crucial tools in understanding cancer's complexities, predicting patient outcomes, and crafting personalized therapeutic approaches. As cancer persists as one of the leading causes of mortality globally, the need to advance early detection, accurate diagnosis, and effective treatment methods remains critical. In this context, *The Potential of Cancer Biomarkers: From Discovery to Clinical Application* offers a comprehensive overview of the essential role biomarkers play in the continuum of cancer management.

This book is divided into several thematic sections, each addressing key areas in biomarker research. Initial chapters introduce the fundamentals of cancer biomarker discovery, outlining the methodologies that enable the identification of novel biomarkers. A major focus of this book is the application of biomarkers in precision oncology. As precision medicine becomes increasingly central to modern cancer care, biomarkers allow for the tailoring of treatment plans that match the specific molecular profile of a patient's tumor. Detailed chapters are dedicated to exploring biomarkers as predictive and prognostic tools, encompassing a range of cancer types. Additionally, new developments in biomarker-guided immunotherapies, liquid biopsies, and multiomics approaches are discussed, underscoring the dynamic intersection of technology and clinical practice. While advances in biomarker research offer immense promise, translating these discoveries from bench to bedside is a complex process. This book addresses practical considerations in biomarker deployment, from regulatory hurdles and clinical trial design to data interpretation and integration into standard care. Through case studies and real-world applications, readers gain insights into how biomarker research is shaping current and future cancer therapies.

The Potential of Cancer Biomarkers: From Discovery to Clinical Application is designed for researchers, clinicians, and healthcare professionals who seek to deepen their understanding of biomarker science and its clinical applications. Each chapter provides a window into the multifaceted world of cancer biomarkers, aiming to equip readers with the knowledge needed to drive impactful research and enhance patient care. The collective expertise of contributing authors offers both a broad view and detailed insights, making this book a valuable resource for advancing biomarker research in oncology.

This work aims to bridge the gap between scientific discovery and clinical application, presenting the latest advancements, challenges, and future directions in cancer biomarker research. We hope this book not only informs but also inspires continued innovation in the fight against cancer.

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CHAPTER

1 Introduction: navigating the landscape of cancer biomarkers

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1.1 Introduction to cancer—a global health issue

The World Health Organization (WHO) defines cancer as a generic term covering a wide range of diseases which is characterized by uncontrolled cell division that can spread to other organs in the body. About 100 different types of cancer and a far bigger number of cancer subtypes have been found, all of which have converged on certain critical characteristics that drive cancer progression. These include sustaining proliferative signaling, evading growth arrest, suppressing apoptosis, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism, and evading immune destruction. Genetic instability and tumor-promoting inflammation have been regarded as the enablers of cancers (Hanahan & Weinberg, 2011).

1.2 The beginnings of cancer—carcinogenesis

Cancer development is a multistage process known as carcinogenesis. The different stages in carcinogenesis are driven by a carcinogen-induced genetic or epigenetic damage in cells that gain a selective growth advantage and undergo clonal expansion due to the activation of proto-oncogenes and inactivation of tumor suppressor genes. The different stages of carcinogenesis include- initiation, promotion, and progression (Malarkey et al., 2013). Initiation involves the

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alteration, change, or mutation of genes of normal cells in response to exposure to a carcinogenic agent. The promotion stage consists of the proliferation of initiated cells and enhances the probability of accumulation of further genetic mutations that drive the further progression of the tumor. Within this stage, chemopreventive agents can still alter the process. Progression is the final stage of neoplastic transformation, where further genetic and phenotypic changes occur. This involves a fast increase in the tumor size, where the cells may undergo further mutations with invasive and metastatic potential. Metastasis involves the further spreading of cancer cells through the connective tissues to other parts of the body (Siddiqui et al., 2017).

1.3 The common types of cancer

Some of the major forms of cancer showing a high rate of incidence and causing a high rate of death globally are lung cancer, liver cancer, breast cancer, colorectal cancer, and prostate cancer.

1.3.1 Lung cancer

Lung cancer is a global burden leading to disabilities and premature mortality. Symptoms of lung cancer include coughing, dyspnea, hemoptysis, lung infection, shortness of breath, bone pain and tenderness, loss of appetite, muscle weakness, fatigue, weight loss, and anorexia (Latimer & Mott, 2015). There are several carcinogens that can cause lung cancer. As per IARC data, smoking or intake of tobacco is the major contributor to lung cancer and is the reason behind over 80% of the cases of lung cancer. Lung cancer is classically divided into two different types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (Herbst et al., 2008). The major driver alterations in lung cancers include *EGFR* (epidermal growth factor receptor) and *KRAS* (Kirsten rat sarcoma viral oncogene) mutations along with *EML4-ALK* (Echinoderm microtubule-associated protein-like 4- anaplastic lymphoma kinase) fusions. However, mutations in *ERBB2* (Erb-b2 receptor tyrosine kinase 2), *MAP2K1* (mitogen-activated protein kinase 1), and *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) have also been reported in a few cases. Certain alterations like mutations in *TP53* (Tumor suppressor protein 53), *BRAF* (B-Raf proto-oncogene), *PIK3CA*, *MET* (MET proto-oncogene) and *STK11* (serine/threonine kinase 11), loss of *PTEN* (phosphatase and tensin homolog), and amplification of *MET* (MET proto-oncogene) and *MYC* (MYC proto-oncogene) are common in NSCLC (Pikor et al., 2013). NSCLC is of three different histologic types: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. Squamous cell carcinoma is associated with smokers, while adenocarcinoma is associated with non-smokers. NSCLC comprises 85% of lung cancers and adenocarcinoma has the highest rate of incidence amongst the NSCLC histology types (Frisone et al., 2022; Herbst et al., 2008).

1.3.2 Liver cancer

Liver cancer or hepatocellular carcinoma (HCC) is one of the top five cancers in the world in terms of occurence and mortality. The most frequent driver gene changes seen in nearly 80% of HCC patients include telomerase activation via telomerase reverse transcriptase (TERT) promoter mutation, viral insertion, chromosomal translocation, or gene amplification (Jang et al., 2021). Oncogenes and tumor suppressor genes are the most significant group of genes and are frequently changed in HCC. According to studies, TP53, RB1 (RB transcriptional corepressor 1), CCNA1 (Cyclin A1), CCNE1 (Cyclin E1), and PTEN all have frequent mutations or genetic changes that affect cell cycle control (Tornesello et al., 2016). The chromosomal amplification of the MYC or MET, CCND1 (Cyclin D1), or FGF19 (Fibroblast growth factor 19) genes has also been altered, resulting in their overexpression and the activation of numerous oncogene signaling pathways (Huang et al., 2018). Chronic inflammation is thought to be one of the primary initiators of HCC, carcinogenesis, and tumor growth since it is characterized by the invasion of macrophages and immature myeloid cells as well as dysregulated cytokine production (Yu et al., 2018). The release of cytokines and growth factors that support tumor cell proliferation or counteract apoptosis, as well as paradoxically reducing the anti-tumor function of nearby lymphocytes, are the two main pro-tumorigenesis methods by which immune cells contribute to HCC (Quail & Joyce, 2013). The primary inflammatory signaling pathways implicated in promoting HCC are the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and JAK-STAT (Janus kinase- signal transducer and activator of transcription) pathways (Morris, Mortimer and O'Neill, 2022).

1.3.3 Breast cancer

Breast cancer has the highest rate of incidence among all cancers in women and also leads to the highest number of cancer-related deaths in women. Breast cancer is a complex disease with genetic and environmental factors involved in it. The two major susceptibility genes in breast cancer are *BRCA1* (breast cancer-associated gene 1) and *BRCA2* (breast cancer-associated gene 2), both of which are involved in the maintenance of the genome and DNA repair (Fu et al., 2022). More recently, mutations in certain other genes have been found to be involved in breast cancer development. These include: *TP53*, *PTEN*, *STK11*, *CHEK2*, *ATM* (ataxia telangiectasia mutated), *PALB2* (Partner and Localizer of BRCA2), and *BRIP1* (BRCA1 interacting protein C-terminal helicase 1) (Apostolou & Fostira, 2013). The classical immunohistochemical markers of breast cancer are ER (estrogen receptor), PR (progesterone receptor), and HER2 (human epithelial growth factor receptor 2). These markers, together with clinicopathological variables including tumor size, tumor grade, and nodal involvement, are conventionally used for patient prognosis and management.

1.3.4 Colorectal cancer

Colorectal cancer has the third highest rate of cancer incidence. Major contributory reasons for the development of this disease include genetic factors, intestinal inflammatory diseases, polyps, and smoking. Colorectal cancers comprise a heterogeneous group of diseases with chromosome instability, microsatellite instability, aberrant DNA methylation, and DNA repair defect's significant role in colorectal epithelial cell transformation leading to colorectal cancer (Granados-Romero et al., 2017). Deregulation of several signaling pathways like PI3K/Akt, Wnt (wingless-type MMTV Integration), and NF-κB also contributes to progression and resistance of colorectal cancer to treatment. 5-Fluorouracil and leucovorin in combination with oxaliplatin or treatment with anti-EGFR or anti-VEGFR (vascular endothelial growth factor receptor) antibodies are the current therapeutic modalities for dealing with colorectal cancer (Martini et al., 2017).

1.3.5 Prostate cancer

Prostate cancer is the highest diagnosed cancer in men. The biochemical marker of prostate cancer is a prostate-specific antigen. The first prostate cancer susceptibility gene identified was HPC1 gene which happens to be a defense against viral infections indicating viral infection as an epigenetic factor for causing prostate cancer. The main driver of prostate cancer is the androgen receptor, which has many splice variants, and a few of them have shown tumor-promoting ability. Targeting the androgen receptor leads to tumor regression which can lead to growth of prostate cancers that are independent of androgen receptor for their growth and thus resistant to androgen targeted drugs (Lamb & Neal, 2013). Although the major oncogenes such as *RAS*, *MYC,* among others seem to have no major links with prostate cancer, several tumor suppressor genes like *TP53*, *PTEN*, *BRCA*, and DNA repair defects have been associated with prostate cancer (Sumanasuriya & De Bono, 2018) (Fig. 1.1).

1.4 Introduction to biomarkers

The term "Biomarker" is a broad term used to define various clinical parameters that allow the correct medical assessment of a patient in an accurate and reproducible manner. The WHO defines biomarkers as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease." Biomarkers can range from blood pressure to sugar levels and the level of expression of various genes. Biomarkers allow us to link between measurable clinical parameters and clinical outcomes. Biomarkers can help speed up the treatment processes and also allow the development of better therapeutic options (Strimbu & Tavel, 2010).

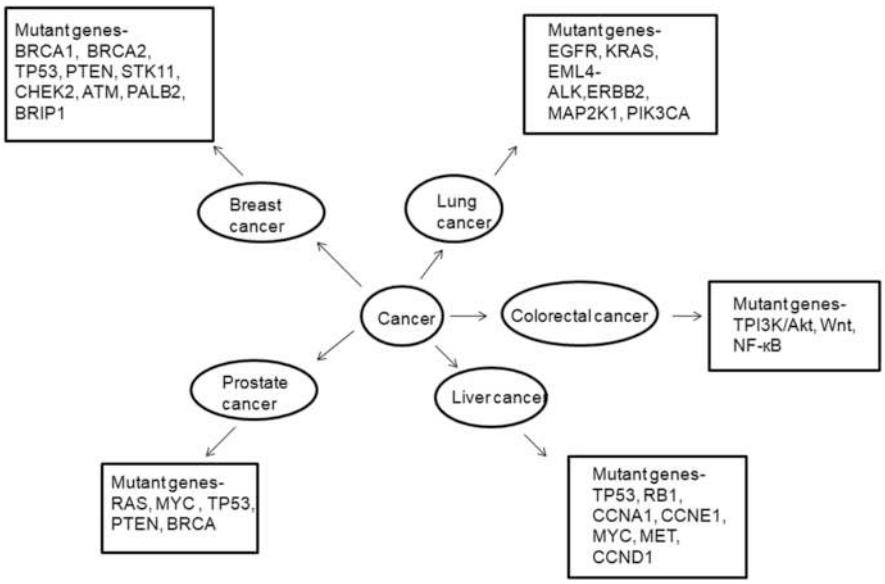

FIGURE 1.1

The major types of cancers and the important mutant genes involved in each cancer.

Biomarkers have played critical roles in the diagnosis and therapeutics of cancers. Most cancer-related biomarkers are present in bodily fluids or tissues. Cancer biomarkers can distinguish between the diseased person, the stage of the cancer, and the response to treatment procedures. Cancer biomarkers can arise due to several reasons including germline or somatic mutations, and modifications at the transcriptional or post-transcriptional levels. The variety of cancer biomarkers ranges from mutant proteins, fusion proteins, overexpressed proteins, noncoding RNAs or the patterns of gene expression, protein expression, or metabolite pattern (Sarhadi & Armengol, 2022). Below we discuss some of the major classes of biomolecules that serve as biomarkers in cancer.

1.5 Categories of biomarkers in cancer

1.5.1 Mutant genes

Mutant genes are often responsible for the development of cancers. As such, it is imperative that the mutant genes have been studied as biomarkers in cancer. Mutations can happen both at the somatic levels and the germline level. Germline mutations can be passed to the next generation as they occur in the gametes. These mutants can include mutated tumor suppressor genes like BRCA1 and BRCA2. In the case of tumor suppressor genes, the mutations are mostly inactivating mutations

like insertions and deletions, nonsense mutations, and splicing-based mutations. On the other hand, oncogenes suffer activating mutations. Germline mutations have also been reported in several DNA repair pathways like nucleotide excision repair, homologous recombination, and miss-match repair pathways. These defects have been observed across various cancers such as breast, ovarian, urothelial, among others. These mutations in these genes have been labeled as moderate and high-risk cancer-related gene variants (Sarhadi & Armengol, 2022; Vlachostergios et al., 2020).

Somatic genetic mutations are often the major driving forces behind the progression of cancers. Somatic mutations build up along with the progression of cancer. The most common somatic mutations in cancers involve the genes TP53 and EGFR. TP53 mutation has been correlated with the prognosis of several types of cancers. The status of TP53 has also been correlated with a poor response to treatment in several cancers like head and neck cancers, breast cancer, and leukemia. EGFR gene carries a gain-of-function mutation in lung cancers and its inhibition has been the target of drug development (Olivier & Taniere, 2011). A second level of somatic mutations often have been reported in various cancers. These include mutations in the genes of ARIDA1 (AT-rich interactive domain protein 1), CDKN2A (cyclin-dependent kinase inhibitor 2 A), ELF3 (E74 like ETS transcription factor 3), among others. These mutations have been associated with a negative response to treatment procedures (Wardell et al., 2018). Somatic mutations in cancer have been narrowed down to 275 substitutions occurring across 275 genes. Of prominence are mutations in tumor suppressor genes like TP53, PTEN, and the Ras-related small GTPases like RAC1 (RAC family small GTPase) and RRAS2 (RAS-related 2). Certain mutational hotspots arise in genes across different cancers. For example, EGFR Y375C occurs in urothelial and renal cancers, while KNSTRN (Kinetochore Localized Astrin) S24F occurs in squamous cell carcinoma and cutaneous melanoma (Chang et al., 2016). Mutations in genes of TP53, *PIK3CA* H1047, *PIK3CA* E545 mutations, and ESR1 mutations have been reported in over 20 major types of cancers including adenocarcinomas, blastoma, etc. These mutation hotspots have been associated with the onset of high rate of metastasis in cancers (Zehir et al., 2017).

1.5.2 Epigenetic modifications

Epigenetic modifications have been reported in both diseased and healthy circumstances. These modifications often occur in proteins that are associated with the binding of DNA. Epigenetic proteins have been helpful in detecting cancer in its preliminary stages from plasma samples. Epigenetic medications and metabolic modifications occur in an interdependent manner which influences the actions of the genetic markers of cancer ultimately leading to the progression of cancer. Events like methylation, acetylation, etc. are often the major factors in epigenetic modifications. These modifications can happen on the DNA or proteins

like histones. Altered methylation of DNA can lead to erroneous expression of critical genes like tumor suppressor genes leading to cancer initiation and progression. Altered patterns of histone modifications have been noted across various cancers and have corresponded with low levels of response to treatment (Sun et al., 2022). DNA methylation occurs in the Cytosine residues. DNA hypermethylation or hypomethylation can have various effects. DNA hypomethylation often leads to genomic instability, activation of oncogenes, and activation of fetal genes. On the other hand, DNA hypermethylation can have the two pronged effects of silencing tumor suppressor genes while activating oncogenes. Hypermethylation also leaves the DNA more vulnerable to deamination, UV, and chemicals-induced damage. The major enzymes that are responsible for the methylation are the DNA methyl transferase enzymes (DNMT1,2,3). Mutations in these genes have been associated with the development of cancers (Ilango et al., 2020). RNA epigenetic modifications have also been reported and methylations have been associated with all the bases present in RNA. Adenine residues are the most commonly methylated in RNA. Methylation of RNA can influence the half-life of RNA, and it has been observed to cause the lowering of expression of genes (Zhao et al., 2020).

1.5.3 Extracellular circulating nucleic acids

Circulating nucleic acids can be majorly classified into miRNAs and circulating RNA and DNA (CircRNA and CircDNA). miRNAs are short nucleotides that are involved in regulating gene expression of both tumor suppressors and oncogenes among other genes. miRNAs can also act as activators of cell surface receptors. miRNAs are rapidly synthesized; they are specific for the sequences that they will bind and they are available in the plasma. As such, miRNAs are being increasingly regarded as biomarkers. A profile of miRNAs is analyzed to arrive at a proper diagnosis of the type of cancer as different cancers will have different profiles of miRNAs which can serve as a miRNA-based fingerprint. Certain miRNA levels have also been predictive of the response to treatment. For example, miR-21 has been associated with a positive response to treatment in several cancers like leukemia and NSCLC. miR-184 levels have been reported to be high in tongue squamous cell carcinoma and the levels lower with a favorable response to treatment (Kabzinski et al., 2021). miRNA expression patterns are affected in cancers. This can occur due to epigenetic modifications involving methylation of DNA sequences in the promoter regions of miRNAs. Acetylation of histones in cancers like breast cancer can cause the decreased expression of miRNAs involved in regulating oncogenes. Mutant proteins like BRCA1 and EGFR can also influence miRNA expression patterns in cancer allowing the increased expression of pro-oncogenic miRNAs (Bertoli et al., 2015).

CircDNA are important biomarkers in the detection of cancers, especially at the early stages. CircDNA have been useful in the of detection the location of major

cancers of the lung, liver, ovary, and pancreas (Gao et al., 2023). CircDNA can be easily obtained from the bodily fluids hence being easily available for analysis. These DNA molecules carry the methylation patterns along with other epigenetic changes occurring on the cancerous tissues as they originate from the specific tissues. CircDNA levels rise along with the progression of cancer and with increased cell death occurring in the tumor microenvironment. The half-lives of circDNA increase in cancer patients compared to healthy individuals thus making them good prospective biomarkers (Kustanovich et al., 2019). The mutational load in CircDNA can also be analyzed for selecting the appropriate treatment course and also to check the response to treatment (Song et al., 2022). CircRNA are being regarded as potential biomarkers in the detection of type of cancer and its stage and to the response to therapy. Several studies have found relations between the CircRNA profile and the development of solid tumors like prostate, lung and colorectal cancer. CircRNA also provides information on the status of expression of different genes involved in cancer and any potential mutations that the genes might be having (Cheung et al., 2019). Studies have shown that thyroid-stimulating hormone receptor mRNA remains in circulation at higher levels in the body fluids of thyroid cancer patients (Zhang et al., 2017). The CircRNA profiles of both humans and the microbiome are being looked into to diagnose cancer, the tissue of occurrence and the mode of therapy. Checking the microbiome CircDNA along with the human CircDNA has shown better diagnostic accuracy with regard to several solid tumors. Microbes have been known to be causative agents for cancers like stomach cancer, colon cancer along with viral-induced cancers like liver and cervical cancers. Specific microbes have also been reported to survive in the tumor microenvironment of different types of cancers. These studies signify the potential of CircRNA from human and microbiome as biomarkers for the type of cancer, site of cancer, and the response to treatment (Chen et al., 2022) (Table 1.1).

1.6 Features of biomarker genes

In the next section, we discuss the various features of some of the major genes that act as biomarkers across several cancers.

1.6.1 EGFR

Epithelial growth factor receptor (EGFR) is a transmembrane glycoprotein with an extracellular growth factor receptor domain, a transmembrane domain, an intracellular tyrosine kinase domain, and a regulatory domain that regulates several pathways like RAS (rat sarcoma viral oncogene)-RAF (V-Raf-1 murine leukemia viral oncogene)-MEK (dual specificity mitogen-activated protein kinase kinase)-ERK (EPH receptor B2), PI3K-AKT-mTOR and JAK-STAT which promote cell proliferation, metastasis, and suppression of apoptosis (Bethune et al., 2010). There

Table 1.1 Different categories of biomarkers and their potential clinical uses.

Type of biomarker	Examples	Potential clinical uses	References
Germline mutant genes	TSC2 (TCS complex subunit 2), HLA-A (major histocompatibility complex), TET2 (TET methylcytosine dioxygenase 2), GEN1 (GEN1 holiday junction 5' flap endonuclease), NCOR2 (nuclear receptor corepressor 2)	Biomarkers for prediction of toxicity arising out of radiation therapy against head and neck squamous cell carcinoma	Deichaite et al. (2022)
	CDKN2A, CDK4 (cyclin-dependent kinase 4), ATM, POLH (DNA polymerase eta), XPC (xeroderma pigmentosum)	Biomarkers for predicting the response to systemic therapy in cutaneous melanoma	Aoude et al. (2020)
Somatic mutant genes	SF3B1 (splicing factor 3b subunit 1), ASXL1 (ASXL transcription regulator 1)	Biomarkers for predicting the survival and response to therapy in B-cell lymphoma	Eide et al. (2010)
	SPEN (SPEN family transcriptional repressor)	Biomarker for predicting response to immunotherapy in B-cell lymphoma, pancreatic carcinoma	Li et al. (2023)
	SYNE1 (Spectrin repeat nuclear envelope protein 1), *FOXA1* (Forkhead Box A1), *LRP1B* (LDL receptor- related protein 1B), *FAT3* (FAT Atypical Cadherin), *SPOP* (Speckle-type BTB/POZ protein)	Biomarker for predicting tumourogenesis in prostate cancer	Mamidi et al. (2019)
Epigenetic modifications	DNA modifications- Hypermethylated SEPT9 (Septin 9) and MGMT (O-6-methylguanine-DNA methyltransferase) genes	Biomarker for diagnosis of colorectal cancer and glioblastoma respectively	Locke et al. (2019)
	Methylated RNA-associated with high levels of METTL3 (methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit), YTHDF1 (YTH *N*-methyladenosine RNA binding protein F1)	Biomarkers for poor survival and low response to therapy in breast cancer	Zheng et al. (2021)
Extracellular circulating nucleic acids	miRNA- miR-129-1-3p and miR-566, miR-23a and miR-1246	Biomarkers for early detection of colorectal and colon cancer	Chakrabortty et al. (2023)
	Circulating DNA- mutant KRAS DNA segment and mutant PIK3CA DNA	Biomarkers for prediction of resistance to therapy in colorectal cancer and response to therapy in breast cancer, respectively	Duffy and Crown (2022)
	Circulating RNA- hsa_circ_0006117 and hsa_circ_0066147	Biomarkers for early detection of pancreatic ductal adenocarcinoma	Xu et al. (2024)

is overexpression of EGFR or mutations in the intracellular domains of *EGFR* in a majority of cancer cases. The majority of the mutations in *EGFR* are concentrated on the tyrosine kinase domain with deletions in exon 19 and point mutations in exon 21 occurring in over 90% of the lung cancer cases, while a few cases of frame insertion mutation in exon 20 have also been reported (Hsu et al., 2018). *EGFR* gene also undergoes amplifications and in several cases of adenocarcinoma, there was both an increase in gene copy number of *EGFR* and mutation in *EGFR,* while in other cases, there was only one of the conditions. In the majority of cancer cases, with *EGFR* gene amplifications, there was also the presence of *EGFR* mutations (Peng, Liang, et al., 2022).

The central role of EGFR in development of cancer has promoted the targeting of EGFR as a therapeutic intervention in various cancers like NSCLC. A majority of them target the tyrosine kinase domain of EGFR and are known as tyrosine kinase inhibitors (TKIs), while another drug type includes monoclonal antibodies directed against the extracellular domain of EGFR. Currently, two TKIs, Gefitinib (Iressa), and Erlotinib (Tarceva) are used as a first line of treatment against advanced and metastatic cancer (Forcella et al., 2017). The use of these TKIs improved survival in patients with mutations in exon 19 and exon 21 of *EGFR* gene and they performed better than conventional chemotherapy. However, these two TKIs also have limitations as they cannot prevent the cancer growth in NSCLC cases with mutations in exon 20 of *EGFR* (Liu et al., 2017). This led to the development of second-generation TKIs, like afatinib and dacomitinib, which could inhibit EGFRs with mutation in exon 20 but in clinical trials, both the drugs showed no significant clinical activity (Hsu et al., 2018). Clinical trials are now going on with third generation of TKIs, such as Osimertinib, which have a low selectivity for wild-type EGFR and have a high selectivity for EGFR with mutations in exon 20. However, in the clinical trials, there was resistance against osimertinib but the mechanisms of the acquired resistance still have not been uncovered (Hirsh, 2018). The use of TKIs has its own share of undesirable side effects, which includes diarrhea, acneiform skin rash, paronychia, mucositis, stomatitis, cornea erosion, epistaxis, interstitial lung disease, and liver function abnormalities (Hsu et al., 2018). Monoclonal antibody against EGFR that is being clinically tested is cetuximab; however, it has not moved beyond clinical trials (Liu et al., 2017).

1.6.2 KRAS

The *RAS* gene family includes *HRAS*, *KRAS*, and *NRAS* and encodes for GTP-binding proteins of 21 kDa and they control the activation of several pathways involved in cell proliferation and apoptosis such as MAPK (mitogen-activated protein kinase 1), PI3K, and STAT. RAS happens to be integration point for upstream growth factor signals received by the growth factor receptors and downstream signaling pathways (Cuesta et al., 2021). Mutations in all the three types of RAS have been associated with cancer; however, mutations of KRAS have

mostly been reported in lung cancer. Mutations in KRAS have mostly occured in the adenocarcinoma histologic variety of lung cancer and never in squamous cell carcinoma. Mutations majorly occur in codon 12 of *KRAS,* while mutations in codon 13 and 61 have also been reported (Garrido et al., 2017).

KRAS is a negative prognostic factor for NSCLC treatment. NSCLCs with a wild-type KRAS have been reported to respond better to treatment compared to NSCLC with mutated NSCLC (Goulding et al., 2020). Although KRAS is widely mutated in several types of NSCLCs, there is still no specific drug to act on the KRAS-induced signaling so that there is a better control of NSCLC. The failure of drugs targeted at the downstream targets of RAS to yield any favorable outcome has put the focus back on RAS to be the target of potential new drugs against NSCLC. There are several groups trying to target the GTPase domain of RAS and most of them are work in progress. Of particular, interest to most of these groups is the KRAS G12C mutant which is the major mutant form of KRAS in NSCLC (Ostrem & Shokat, 2016). Although KRAS has stayed undruggable, the status quo may change in the near future.

1.6.3 TP53

The *TP53* gene encodes for the p53 protein which is also known as "the guardian of the genome" and is frequently mutated in cancers. It can also suffer from oncogenic "gain of function" mutation. p53 protein is a DNA-binding transcription factor that is involved in DNA repair, metabolism, cell cycle arrest, apoptosis, and senescence (Chen et al., 2022). p53 is not only critical in repairing DNA damages but also activated by ARF in response to oncogenic signals. p53 promotes apoptosis in cells having the oncogenic signals. In fact, it has been observed that DNA repair and tumor prevention by p53 are independent of each other and the tumor prevention function of p53 was largely dependent on ARF-dependent p53 activation (Carrasco-Garcia et al., 2017).

The common mutations occurring in *TP53* gene in NSCLC and other cancers involve missense mutations in 80% of cancers with frameshift insertions and deletions, nonsense mutations, and silent mutations making up for the rest of the reported mutations in *TP53* in cancer (Chen et al., 2022). Phenotypes associated with p53 "gain-of-function" mutations include increased tumourigenicity, increased growth rate and motility, increased metastasis and invasiveness, increased growth in soft agar, and increased resistance to chemotherapeutic drugs. Mutant p53 modulates the levels of several genes; for example, mutant p53 upregulates multidrug resistance proteins, proliferating cell nuclear antigen (PCNA), EGFR, c-MYC, IGF, cyclin A, and cyclin B1; downregulates the proapoptotic CD95/Fas/Apo1, caspase-3, and PTEN; and also prevents the formation of the MRN (Mre11-Rad50-Nbs1) complex, thus preventing it from binding to DNA breaks (Oren & Rotter, 2010). The wild-type p53 is a rather unstable protein, whereas the mutant p53 is more stable. Unlike the wild-type p53 which can transactivate *MDM2*

(MDM2 Proto Oncogene), which in turn causes the degradation of wild-type p53, the mutant p53 does not transactivate Mdm2, thus promoting stability of the mutant p53 (Terzian et al., 2008).

Targeting the mutant p53 and restoring its wild-type function has been a major focus point of p53 targeted therapeutic drug research and several undergoing research works have shown promise (Kogan & Carpizo, 2016). Several peptides are under study to check for their ability to bind the mutant p53 and restore its wild-type functions. These peptides are referred to as p53 conformation activating peptides. Fungal extracts, dietary extract phenethyl isothiocyanate, and zinc metallochaperones have also been reported to restore the wild-type activity of mutant R175H p53 by promoting the binding of Hsp40 with p53 (Hiraki et al., 2015).

1.6.4 MYC

In mammalian cells, MYC (myelocytomatosis oncogene) proteins arise from three different gene family members: *CMYC*, *LMYC*, and *NMYC*, all of which have a similar function, but with varying potency and patterns of expression. c-Myc is mostly expressed in blood-borne and solid tumor. N-Myc is frequently overexpressed in solid cancers of neural origin. L-Myc is mostly expressed in SCLC (Tansey, 2014). Amplification of c-Myc has been observed in different types of cancers like gastric cancer, colon cancer, breast cancer, and NSCLC (Liu et al., 2016). Myc activation can result in increased cell proliferation and growth, heightened DNA replication, increased protein biogenesis, increased angiogenesis, and metastasis. Myc is involved in carcinogenesis in association with other oncogenic insults like abrogation of the cell cycle control, suppression of apoptosis, and exposure to toxins or carcinogens, autocrine factors, cytokines like TGF α (transforming growth factor alpha), and innate immunity to initiate cell plasticity so that the cells acquire stem cell-like properties and initiate cell proliferation (Gabay et al., 1424). For example, Myc has been shown to act in tandem with loss of wild-type p53 to induce proliferation and tumourigenensis in adult hepatocytes (Beer et al., 2004). Myc promotes tumors via suppression of both innate and adaptive immune response and immune regulatory cytokines. Myc achieves so via promoting expression of CD47 and PD-L1, which are immune suppressors. Myc inactivation could promote tumor regression by restoration of immune responses (Kortlever et al., 2017).

Owing to the nuclear location of Myc, lack of a defined ligand binding site, and physiological functions critical to normal tissues, Myc has more or less remained "undruggable" (Whitfield et al., 2017). This has resulted in the development and testing of drugs that target Myc transcription and translation inhibition, Myc destabilization, disruption of Myc/Max complex, as well as synthetic lethality associated with Myc overexpression. Inhibitors have been designed against the proteins BRD4 (bromo domain containing 4), CDK7 (cyclin-dependent kinase 7), and CDK9 (cyclin-dependent kinase 9) which are involved in Myc transcription,

and against PI3K pathway members to prevent Myc translation, and also against Myc/Max dimerization, and Myc/Max DNA binding (Chen, Wu, et al., 2014).

1.6.5 SRC

The *SRC* gene encodes for the Src tyrosine kinase protein and is a gatekeeper for many signaling pathways that are involved in tumor initiation and promotion. Thus Src upregulation is associated with critical roles in tumor growth, metastasis, and angiogenesis. Increased levels of Src expression have been observed in various cancers. Elevated expression of Src has been observed in 100% cases of SCLCs and 60%–80% of lung adenocarcinomas and 50% of lung squamous carcinomas. Src levels are also upregulated in frequent smokers (Wang & Liu, Wang, et al., 2018). Src is frequently upregulated in cancers with mutant EGFR as compared to cancers with wild-type EGFR (Zhang et al., 2007). Src forms a complex with FAK (Focal Adhesion Kinase) and is involved in recruitment of substrates such as CAS, paxillin, and p190RhoGAP which are involved in the reorganization of the actin cytoskeleton and migration. Src acts via the ERK and PI3K pathways to promote the activity of MMPs to carry out metastasis (Antoniadis & Michopoulou, 2011). Src can directly activate the p110α catalytic subunit of PI3K and negatively regulate PTEN which is the inhibitor of the activation of PI3K pathway. Src can also directly activate Akt in a PI3K-independent manner (Mahajan & Mahajan, 2012). Src also activates c-Ras that activates the Ras/MAPK/ERK pathway. Src also promotes the activation of STAT3 which is a transcription factor that promotes the expression of anti-apoptotic Bcl-2, Bcl-X_L proteins, and the tumor promoter c-Myc. Src also promotes the formation of angiogenic cells via the activation of STAT3 (Chen, Elfiky, et al., 2014). Src also upregulates the expression of drug resistance-associated genes causing chemotherapy and radiation resistance in cancer cells (Zhang et al., 2014). Inhibition of Src is also critical in upregulating response of cancer cells to paclitaxel treatment. Src inhibition promotes paclitaxel induced apoptosis in ovarian cancer cells via upregulation of caspase-3 (Chen et al., 2005).

Keeping in mind the critical nature of Src in cancer there are many ongoing attempts at the development of an Src inhibitor that can be used therapeutically. Some of the undertrial small molecule inhibitors of Src include dasatinib, AZD0530, and SKI-606. Most of the Src inhibitors are targeted against the ATP binding pocket of Src and these inhibitors have been successful in tumor regression. Among the many tested inhibitors, dasatinib has gained FDA approval for use as a drug against cancer (El-Rashedy & El-Din, 2018). However, dasatinib now faces issues of suppression of the immune system as Src is critical in the development and activation of macrophages, dendritic cells, natural killer cells, and B- and T-cells and inhibition of Src thereby compromises the immune system of the individual treated with dasatanib. Src inhibitors are designed against the ATP binding pocket of Src and this leads to nonspecific inhibition of other tyrosine kinases leading to further complications (Paydas, 2014).

1.6.6 PTEN

PTEN was cloned in 1997 through its association with the human cancer susceptibility locus at 10q23 and since then PTEN has attained the status of a genome guardian with a lot of cancers reporting a mutated PTEN status. PTEN is a lipid and protein phosphatase which negatively regulates the PI3K pathway and is a tumor suppressor. (Wan et al., 2011). PTEN function is compromised in many cancers through the occurrence of somatic mutations, transcriptional regulation, post-transcriptional regulation by non-coding RNAs, post-translational modifications, protein–protein interactions, gene silencing, and epigenetic mechanisms. Haplo insufficiency of PTEN can also initiate tumor development (Milella et al., 2015).

A common point mutation in the *PTEN* gene is G129E which abrogates the lipid phosphatase activity of PTEN resulting in prevention of tumor-suppressing activity of PTEN. *PTEN* mutations often occur in cancers alongside PIK3CA mutations (Chalhoub & Baker, 2009). PTEN inactivation also results in increased invasiveness and anchorage-independent growth (Zhu, Yu and Chai, 2016). The position of the mutations also determines the type of mutant that is produced in the case of PTEN. Mutations such as frameshift or a truncation within the first eight exons lead to monoallelic expression by nonsense-mediated decay. Mutations in the ninth codon onward which code for the C terminal tail of PTEN do not disrupt catalytic activity but affect the protein stability and regulation (Leslie & Longy, 2016).

PTEN acts in a dose-dependent manner and in many cancers the loss of activity is mostly partial. This is evident from a study which found several cases of PTEN mutants which retained 50% of catalytic activity but still there was development of tumor (Carracedo et al., 2011). Cancers can express both normal and mutant PTEN. PTEN acts in dimers and the binding of the mutant PTEN to the normal PTEN affects the activity of the normal PTEN which in turn leads to greater activation of the PI3K pathway. The mutant PTEN are more stable than the normal PTEN and that makes mutant PTEN have a longer half-life. Missense mutations of R130G and R130Q are common in many cancers and code for the stable mutant of PTEN (Leslie & Den Hertog, 2014). Inherited *PTEN* mutations can also cause cancer predispositions. *PTEN* mutations have also been reported to coexist with other driver mutations like *TP53* and *EGFR,* while no *PTEN* mutations were reported in NSCLC with *KRAS* mutations. NSCLC with *PTEN* mutations happened mostly in case of smokers (Jin et al., 2010).

1.6.7 PI3K

PI3K mutations occur mostly in the *PIK3CA* gene coding for the catalytic subunit of PI3K and p110α, leading to the hyperactivation of the PI3K pathway. *PIK3CA* mutations are common in many cancers and have been associated with poor prognosis and resistance to treatments (Martínez-Sáez et al., 2020). *PIK3CA*

mutations frequently co-exist with other tumors and some studies indicate a role of *PIK3CA* mutations in promoting resistance to TKIs. *PIK3CA* gene amplifications can coexist with *MET* protooncogene amplifications and such coexistence causes resistance to MET inhibitors (Kang et al., 2017). *PIK3CA* mutations occur in exon 9; E545K; while there are also reports of *PIK3CA* mutations in exon 20 of the *PIK3CA* gene which code for the helical and kinase domains of p110α subunit (Ranjbar et al., 2019). *PIK3CA* mutations occur in the presence of several other mutations in adenocarcinoma, mostly mutant *KRAS* and mutant *EGFR*, and have mostly been regarded as passenger mutations widely distributed with several other mutations and have been considered as not good candidates for specific inhibition. The coexistence of *PIK3CA* mutations along with the driver mutations of *KRAS* and *EGFR* results in poor prognosis of cancers (Scheffler et al., 2015).

The mutations of *PIK3CA* are limited to 3 hotspots, two of which lie in the helical domain and one in the lipid kinase domain, all of which results in an increased lipid kinase activity. *PIK3CA* mutations cause anchorage-independent growth in primary fibroblasts. E542K and E545K are the mutations occurring in the helical domain of PI3K 110α subunit and these mutations interfere with the p85 subunit of PI3K binding and regulating the activity of PI3K 110α which in turn leads to the increased activity of PI3K 110α subunit. H1047R mutation occurs in the kinase domain of PI3K 110α and this induces a conformational change which is mimics the conformational change induced by Ras thereby activating the catalytic subunit even in the presence of an attached p85 subunit (Zhao & Vogt, 2008) (Fig. 1.2).

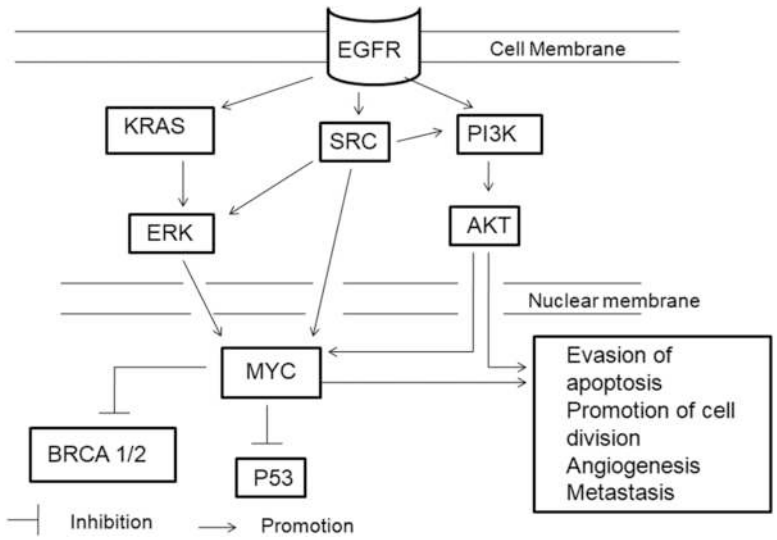

FIGURE 1.2

Figure showing the inter-relations of the major mutant genes in cancers.

1.7 Liver cancer and biomarkers

1.7.1 Underlying causes

The face of liver cancer has transformed drastically over the past century and it is recognized as one of the leading causes of cancer-related death globally. Liver cancer exhibits diverse pathological subtypes, with HCC which rises from hepatocytes constituting the majority of cases, accounting for approximately 75%–85% (Peng, Zhu, et al., 2022). Malignant HCCs can metastasize and cause a life-threatening risk. In addition to HCC, liver cancer also includes intrahepatic cholangiocarcinoma, originating from bile ducts and various mixed types based on pathological classification such as angiosarcoma, hemangiosarcoma, and hepatoblastoma. The early diagnosis of liver cancer is frequently challenging since signs and symptoms typically manifest only in the later stages of the disease. The risk of developing liver cancer is greater in men compared to women with a global incidence ratio of 2.8:1 in men and women, respectively (Kulik & El-Serag, 2019). Diagnosing HCC in children presents even more challenges. Although it is uncommon in this age group, the potential diagnosis of HCC can be a disconcerting prospect for children. The occurrence of liver cancer varies based on geographic and demographic factors and is linked to the prevalence of Hepatitis B within the population (Khanna & Verma, 2018). Indeed, developed countries are witnessing Hepatitis C and alcoholic cirrhosis emerging as notable contributors to HCC. Recently, Non-alcoholic Fatty Liver Disease and Non-alcoholic Steato Hepatitis have also emerged as an established cause of HCC (Huang et al., 2021; Medavaram & Zhang, 2018). However, the geographical distribution of these causes varies globally. Several other associated risk factors like genetic factors, including hereditary liver diseases, may also play a role. Moreover, exposure to aflatoxins, certain chemicals, and environmental toxins can elevate the risk of liver cancer. Obesity and diabetes are recognized as metabolic risk factors, further emphasizing the multifaceted nature of the risk factors associated with liver cancer. There is an ongoing quest for advancements in screening, diagnosis, and treatment approaches to improve health outcomes and help manage liver-related malignancies.

The pathogenesis of HCC is complex and encompasses various molecular abnormalities, including deregulation of the cell cycle, modification in DNA methylation, chromosomal instability, immunomodulation, Epithelial-to-Mesenchymal transition, abnormal regulation of HCC stem cells and dysregulation of microRNA (Chidambaranathan-Reghupaty et al., 2021; Ogunwobi et al., 2019). Late-stage HCC diagnoses often limit their management to palliative care, emphasizing the importance of early detection to improve survival and quality of life. The biological behaviors of these cancers may vary and sometimes be confusing, but laboratory biomarker testing offers a dependable and precise method to diagnose them early. In the context of liver cancer, biomarkers are measurable indicators that provide valuable information about the disease's presence, progression or response to treatment. The early-stage identification of HCC through a combination of imaging and biomarkers has the

potential to enhance patient outcomes significantly. The primary biomarker extensively used in monitoring HCC is serum alpha-fetoprotein (AFP). Structurally, AFP is very similar to albumin which can bind and transport numerous ligands like steroids, heavy metals, bilirubin, drugs, etc. (Wang & Zhang, 2020). AFP is a fetal mammalian glycoprotein synthesized in the yolk sac, fetal liver, and gastrointestinal tract during pregnancy encoded by the AFP gene on chromosome 4q25. After birth, the level of AFP, which has a short half-life of 4–5 days, declines rapidly (Wang & Wang, 2018; Wong et al., 2015). During liver injury, the level of APF again rises significantly in the patient's bloodstream, serving as a supportive biomarker. However, not all HCC cases will have elevated AFP. Indeed, elevated AFP can also be considered in some other benign or malignant conditions. AFP consists of three isoforms: L1, L2, and L3 divided based on binding affinity with Lens culinaris agglutinin (Yi et al., 2013). The L1 isoform is commonly linked to non-HCC, while elevated L3 isoform serves as a dependable tumor biomarker for HCC due to its high sensitivity found in previous meta-analysis studies (Zhou et al., 2021). The evidence implies that the AFP alone is not a foolproof surveillance tool for detecting HCC at an earlier stage, however, useful in distinguishing the condition of benign and malignancy.

1.7.2 Noninvasive biomarker of liver cancer

All cases of HCC do not exhibit elevated AFP levels, emphasizing the need for a multifaceted approach. Currently, there is a rising focus on non-invasive approaches for liver biopsy to overcome the limitations associated with invasive procedures. Non-invasive biomarkers for liver cancer play a pivotal role in the detection, diagnosis and monitoring of this complex and potentially life-threatening condition. Des-gamma-carboxy prothrombin (DCP) is another biomarker that holds significant importance in the context of HCC. DCP is an abnormal form of prothrombin, a protein involved in blood clotting and is characterized by the absence of gamma-carboxyglutamic acid residues (Weitz & Liebman, 1993). Several research studies indicated that the joint evaluation of des-γ-carboxyprothrombin (DCP) and alpha-fetoprotein (AFP) may exhibit greater sensitivity compared to AFP alone (Song et al., 2013). The use of DCP as a biomarker is particularly advantageous in regions where chronic hepatitis B and C infections are prevalent, as these infections are major risk factors for the development of HCC. Additionally, DCP has shown promise in differentiating HCC from other liver diseases, aiding in more accurate and specific diagnoses. Regular monitoring of DCP levels can also be instrumental in assessing the effectiveness of therapeutic interventions and evaluating the progression or regression of HCC. Glypican-3 (GPC3), a cell surface heparan sulfate proteoglycan, is frequently noted for its elevated expression in HCC (Midorikawa et al., 2003). A typical feature of GPC3 is its specificity for HCC, as it is often absent or minimally expressed in normal liver tissue or any benign liver lesions. Elevated GPC3 expression in tissue samples or increased serum levels are indicative of the presence of HCC. This makes GPC3 a reliable biomarker for

distinguishing HCC from non-cancerous liver conditions. Other biomarkers that have been explored to detect and monitor HCC are Osteopontin, Golgi protein-73, Annexin A2, Soluble urokinase plasminogen activator receptor, Midkine, AXL, Thioredoxins (Tsuchiya et al., 2015). Osteopontin is a glycoprotein involved in various physiological processes, including tissue repair and immune response. In contrast, GP73 is a Golgi transmembrane protein which primarily expressed in biliary epithelial cells and slightly in hepatocytes. However, the level of GP73 exhibited an increased expression level in acute and chronic liver disease with significant upregulation in hepatocytes. Annexin A2 also known as p36 is a calcium-dependent phospholipid-binding protein, observed and overexpression in HCC evolved as a potential biomarker for HCC. Over-expression of ANXA2 is commonly detected in a wide range of cancer cells (Wang & Lin, 2014). ANXA2 is seldom found in normal or infected hepatic tissue but overexpressed in HCC. These biomarkers, either individually or in combination, hold promise for improving the accuracy, detection and monitoring of HCC.

1.7.3 Promising liquid biopsy biomarkers for HCC

Liquid biopsy for HCC early detection represents a revolutionary approach in the field of cancer diagnostics and management. A liquid biopsy involves the analysis of biological fluids, such as blood, urine, or other bodily fluids, to identify biomarkers associated with the presence of cancer. The fundamental focus of liquid biopsy is assessing genetic and epigenetic information extracted from bodily fluids. In the case of HCC, liquid biopsy practices primarily focus on detecting circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), microRNA, squamous cell carcinoma antigens, tumor-associated antigens (TAA), and biomolecules like extracellular vesicles (EVs) and exosomes (Bardol et al., 2024; Wang & Yang, Sun, et al., 2020; Ye et al., 2019).

The ctDNA, which comprises fragments of tumor DNA released into the bloodstream, provides a minimally invasive method for detecting genetic alterations associated with HCC. Through liquid biopsy techniques, ctDNA can be analyzed to identify specific mutations, chromosomal aberrations and other genomic alterations in liver cancer cells. The detection of such genetic changes can provide valuable information about the status and characteristics of HCC. This approach not only aids in the early diagnosis of HCC but also facilitates monitoring of disease progression, treatment response, and the emergence of resistance mutations. Circulating tumor DNA typically originates from necrotic and apoptotic tumor cells and constitutes a minor fraction of total cell-free DNA (cfDNA) (Alix-Panabières et al., 2012; Ye et al., 2019). However, the exact mechanism of ctDNA release into the circulating body fluid is not fully known. Examining and evaluating circulating tumor cells (CTCs) released into the bloodstream can also provide valuable information about the nature of the tumor, its aggressiveness, and its potential for metastasis. CTCs are neoplastic cells released from primary tumor sites

which can also serve as a strong predictor of HCC survival (Pantel & Speicher, 2016; Prasoppokakorn et al., 2022). Unlike ctDNA, the presence of CTC in the bloodstream is associated with the viable tumor whose rising level is a clear indication of tumor spread, recurrence, and metastasis process.

Dysregulation of miRNA can alter the genetic architecture of cells and may lead to cancer progression due to enhancing cell proliferation. The human genome is predicted to encode approximately 2600 microRNAs, with over a hundred of them significantly associated or dysregulated during HCC development. Circulatory microRNA can serve as a valuable biomarker for detecting HCC and various other cancers. miR-122 is the predominant liver-specific microRNA, making up over 70% of the total microRNA content within hepatocytes (Elmouttaleb et al., 2015). Multiple studies reported that the downregulation of miR-122a is significantly associated with HCC (Gramantieri et al., 2007; Kutay et al., 2006; Lin et al., 2008). Specifically, HBV-related HCC cases exhibit reduced expression of serum miR-122 when compared to benign cases and control groups (Giray et al., 2014). Other downregulated miRNA associated with HCC are miR-223, miR-1, miR-7, miR10, miR21, miR-26a, miR-29a, miR-33b, miR-34a, miR98, miR-99a, miR-100, miR-101, miR-22, etc. (Morishita et al., 2021). Oncogenic miRNAs are upregulated in HCC development which also serves as a potential biomarker for disease detection. miR-21, an oncogenic promotes fibrosis significantly upregulated in HCC in both tissue and serum (Guo et al., 2017; Zhang et al., 2020). Other upregulated miRNAs associated with HCC development are miR-10a, miR-10b, miR-18a, miR-22, miR-23a, miR-25, miR-30d, miR-106b, miR-107, miR-143, miR-151, miR-155, etc. A list of different dysregulated microRNAs associated with HCC development is listed by Sun et al. (2013) and Morishita, A. et al (2021) in their publication (Sun et al., 2013; Wortzel et al., 2019). Alteration of microRNA expression significantly contributes to the HCC development. Combining the evaluation of both tumor-suppressor and oncogenic microRNA biomarkers has the potential to improve the accuracy of HCC detection and enhance the management of HCC. However, challenges still exist in standardizing liquid biopsy procedures, ensuring sensitivity and specificity and determining the clinical significance of identified biomarkers. Ongoing research is focused on refining liquid biopsy techniques, validating their clinical utility, and integrating them into routine HCC screening and monitoring protocols.

The immunodiagnostic technique can target antigens specific to the disease using specific biomarkers. Exosomes and tumor-associated antigens can also be used as biomarkers to assess HCC. Exosomes are 40–100 nm extracellular vesicles present in diverse body fluids that contain a variety of cellular components (Wang et al., 2017). Tumor cells that secrete abundant biomolecules and proteins suggest that monitoring exosomes could serve as a promising biomarker for cancer including HCC. Adenylate cyclase-associated protein-1 (CAP1) is one of the crucial exosomes derived from HCC cells exhibiting a high metastatic potential (Hu et al., 2015). In contrast, past studies revealed that exosomes originating from

highly malignant HCC cell lines exhibit elevated concentrations of MET proto-oncogenes, receptor tyrosine kinases (MET), S100 calcium-binding protein A4 (S100A4), S100A10, S100A11, caveolin-1 (CAV1), and caveolin-2 (CAV2) (Houghton, 1994; Liu et al., 2023). Exosomes not only offer a versatile platform for the diagnosis of HCC but also function as carriers for drug delivery.

The immune system of humans can identify changes in antigens on cancer cells and produce autoantibodies against these altered antigens, known as tumor-associated antigens (TAA) (Chang et al., 2020). The p53 is among the extensively studied TAA, and anti-p53 antibodies have been recognized for their potential as biomarkers for HCC (Dai et al., 2013). Elevated levels of anti-p53 antibodies have been associated with HCC, providing a specific and measurable indicator for detecting the disease. Some other novel TAA biomarkers are HCC1/CAPER, p62/IMP2(IGF-II mRNA binding protein), p90/CIP2A (Cancerous Inhibitor of PP2A) considered to be significantly associated with the HCC (Ma et al., 2022). Moreover, a recent study identified DNA methyltransferase 3 A(DNMT3A), p16, Hear shock protein-60 (Hsp60) and Heat shock protein A5 (HSPA5) as potential TAA biomarkers associated with Hispanic HCC (Murphy, Weaver and Janeway's, 2016). The recognition of TAAs as diagnostic method facilitates early detection of HCC. Monitoring these antigens not only aids in diagnosis but also holds potential for assessing disease progression and tailoring treatment strategies.

1.7.4 Prospects of HCC biomarkers

The prospects of novel biomarkers in the diagnosis and management of HCC represent a pivotal pace in advancing the precision and efficacy of clinical strategies. As research continually unveils the intricate molecular landscape of HCC, identifying robust biomarkers becomes imperative for early detection and personalized treatment. Emerging biomarkers, such as circulating tumor DNA, microRNAs, genetic markers and biomolecules offer promising avenues for enhanced sensitivity and specificity in HCC diagnosis. However, due to the complexity of this disease and its multifactorial etiology, it is often challenging to identify a single predominant biomarker for HCC identification. Indeed, constant efforts are being continuously made to identify panels of biomarkers or molecular signatures that collectively provide a more accurate representation of HCC. Additionally, future directions in HCC management involve incorporating cutting-edge technologies, including artificial intelligence and machine learning, to analyze complex datasets and refine diagnostic accuracy. Integrating these novel biomarkers into diagnostic algorithms can revolutionize screening programs, enabling earlier detection and intervention, thereby improving overall patient outcomes. In essence, the convergence of novel biomarkers and technological advancements holds immense promise for transforming the landscape of HCC diagnosis and thereby treatment, ultimately fostering effective patient care.

1.8 Antigens and biomarkers

1.8.1 Tumor-associated antigens as cancer biomarkers

Tumor antigens have garnered increasing attention due to their potential utility in cancer management. The term "antigen" denotes the molecular configuration perceived by antibodies or any molecule, including linear molecular fragments derived from the processing of the native antigen, capable of being recognized by T cell receptors (TCRs) (Peri et al., 2023). The concept and significance of tumor antigens have been under discussion since the 1940s and were documented prior to the discovery of T cells, which took place in the 1960s (Miller, 2004; Peri et al., 2023). In a groundbreaking experiment in 1943, Gross (1943) conducted a seminal study illustrating the immune system's role in tumor rejection. In essence, mice were exposed to methylcholanthrene, leading to tumor formation. Subsequent removal of the tumors followed by implantation of tumor cells back into the mice resulted in rejection of the secondary tumors. This experiment demonstrated the induction of acquired immunity specifically targeted against the tumor, independent of genetic disparities between the inoculated mice and those generating the tumor cells. Subsequently, Boon and colleagues replicated similar outcomes using mutagen-treated murine cell lines that were unable to develop tumors in syngeneic mice, thereby confirming that tumors express antigens recognizable by cytotoxic T lymphocytes (CTLs) and affirming the role of the immune system in combating malignant cells (Boon & Kellermann, 1977; Van Pel & Boon, 1982). However, the precise molecular identity of the antigens expressed by tumors and recognized by CTLs was not elucidated until 1989 by Lurquin et al. (1989). The researchers identified a solitary peptide recognized by CTLs that deviated from the self-protein due to a single point mutation. This observation unequivocally demonstrated that tumors, through mutation, express modified proteins, thereby marking the cells for recognition by CTLs (Vigneron & Van Den Eynde, 2011). Presently, based on the expression pattern of the parental gene, tumor antigens can be categorized broadly into and tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs) (Hollingsworth & Jansen, 2019).

1.8.2 Tumor-specific antigens

TSAs are molecules exclusively expressed on the surface of cancer cells and not present in normal tissues. These antigens arise from somatic mutations, viral oncogenes, or cancer-testis antigens. Examples include mutated oncoproteins, neoantigens, and viral antigens. TSAs constitute a unique class of antigens confined to tumors, devoid of presence in healthy cells, stemming from either malignant mutations or viral element activation. Neoantigens, oncoviral antigens, and endogenous retroviral elements (ERVs) fall within this category (Hollingsworth & Jansen, 2019; Rooney et al., 2015; Vigneron, 2015).

Neoantigens, a subset of TSAs, arise directly from genetic modifications triggered by tumor DNA mutations, such as non-synonymous single-point mutations, frameshifts, or insertions/deletions, and are customarily specific to each patient (Schumacher et al., 2019). In 1995, Coulie et al. (Miller, 2004) reported the pioneering instance of a neoantigen, identified as melanoma ubiquitous mutated (MUM-1), which stemmed from a single-point mutation in melanoma cells, resulting in an amino acid alteration within the HLA-B44 restricted nonapeptide. Subsequently, other neoantigens, including mutations in β catenin and CDK4 genes, have been identified; each recognized by autologous cytotoxic T lymphocytes (CTLs) and impacting cellular processes (Mandruzzato et al., 1997; Robbins et al., 1996; Wölfel et al., 1995). In cancer immunotherapy, neoantigens offer inherent advantages as non-self-antigens, thus circumventing central tolerance mechanisms, rendering them highly immunogenic (Schumacher et al., 2019). However, their principal drawback lies in substantial variability both within and among tumors, potentially leading to negative selection within tumors and necessitating tailored vaccine development for individual patients, contingent upon the mutational burden (Ilyas & Yang, 2015; Vigneron, 2015).

Oncoviral antigens, originating from viruses driving oncogenic transformations, constitute the source of peptides recognized by T cells within the HLA context (Hollingsworth & Jansen, 2019; Vigneron & Van Den Eynde, 2011). While shared among tumors of similar types due to the commonality of oncogenic viruses, oncoviral antigens lack patient specificity. Prophylactic vaccines primarily focus on eliciting neutralizing antibodies, whereas established tumor treatment targets T cell epitopes (Pils et al., 2007). For example, human papillomavirus and Epstein–Barr virus vaccines target T-cell responses against specific viral proteins associated with various cancers (Ramos et al., 1997; Tashiro & Brenner, 2017).

ERVs, derived from retroviral RNA integration into germ line cells, represent a fraction of the human genome (Bannert et al., 2018). Reactivated in cancer, ERVs become potential targets for cancer therapeutic interventions. Certain ERVs, termed tumor-specific endogenous retroviruses exhibit minimal expression in healthy tissues yet are overexpressed in tumors, presenting as promising targets for cancer immunotherapy (Rooney et al., 2015). Despite their potential, challenges persist in identifying CTL epitopes and understanding HLA-I presentation mechanisms, warranting further research endeavors (Schumacher et al., 2019).

1.8.3 Tumor-associated antigens

TAAs are molecules that are overexpressed, aberrantly expressed, or selectively expressed in cancer cells compared to normal tissues. They often derive from self-antigens with altered expression levels or post-translational modifications. Examples include cancer/testis antigens, oncofetal antigens, and differentiation antigens. TAAs constitute a diverse group, encompassing antigens originating from genes upregulated in tumors, differentiation antigens, and cancer germline/cancer

testis antigens (Hollingsworth & Jansen, 2019; Ilyas & Yang, 2015). Antigens derived from overexpressed genes in tumors represent normal self-proteins, minimally expressed in healthy tissues but significantly upregulated in cancer cells due to their malignant nature. Proteins like EGFR, hTERT, p53, and carbonic anhydrase IX fall into this category, playing crucial roles in cancer cell survival and offering appealing targets for cancer therapy (Ilyas & Yang, 2015). For instance, Gaugler et al. identified renal antigen 1 (RAGE-1), an overexpressed antigen in renal cell carcinoma (RCC), recognized by autologous CTLs, showcasing potential for cancer immunotherapy (Gaugler et al., 1996; Vigneron, 2015). Another example is HER2/NEU, excessively expressed in epithelial tumors such as ovarian and breast tumors, leading to successful treatments with monoclonal antibodies like trastuzumab and peptide vaccines (Clifton et al., 2016; Mittendorf et al., 2019). Moreover, genes associated with apoptosis pathway upregulation in tumors, like survivin and p53, provide sources for T cell epitopes, illustrating promising avenues for therapeutic interventions (Schmidt et al., 2003).

Given their elevated expression in tumors compared to normal tissues and their presence across various tumors, TAAs have been proposed as safe targets for cancer therapies. For instance, chimeric antigen receptor (CAR) T cells, engineered with an extracellular domain derived from antibody variable regions, have shown efficacy in targeting TAAs in an HLA-independent manner, leading to significant clinical outcomes in B cell lymphomas and leukemias (Kochenderfer et al., 2012; Maude et al., 2015; Rosenberg & Restifo, 2015). However, the main challenge in utilizing TAAs for cancer immunotherapy lies in the comprehensive assessment of their expression across different tissues and physiological conditions, posing risks of "on-target, off-tumor" toxicity and autoimmune reactions. For instance, CAR T cells targeting carbonic anhydrase IX in RCC-induced liver toxicity due to CAIX expression in bile duct epithelium, highlighting potential toxicity issues (Lamers et al., 2013). Moreover, overcoming central and peripheral tolerance mechanisms to break immune tolerance toward self-antigens poses another obstacle in developing effective TAA peptide-based vaccines (Pedersen et al., 2013). The identification and implementation of suitable vaccine adjuvants could mitigate these challenges and enhance therapeutic outcomes (Overwijk, 2017).

Differentiation antigens, normal proteins expressed due to specific tissue functions, represent another class of TAAs. For instance, melanoma differentiation antigens like tyrosinase, Melan-A/MART-1, and gp100/Pmel17 have been identified as targets for CTLs in melanoma patients and healthy individuals (Pittet et al., 1999). These antigens have been extensively studied, with tyrosinase and gp100/Pmel17 being among the first antigens recognized in melanoma (Even-Desrumeaux et al., 2011; Kawakami et al., 1994; Pittet & Valmori, 1999). Similarly, differentiation antigens in prostate cancer, such as prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA), have been explored for immunotherapeutic interventions (Olson et al., 2010). However, the risk of autoimmune toxicity remains a concern, as observed in reactions targeting melanocyte antigens and colorectal cancer antigens (Parkhurst et al., 2011). Cancer

germline/cancer testis antigens (CTAs), a vast family expressed in various tumors but absent in normal tissues except for testis and placenta, present promising targets for cancer immunotherapy (Almeida et al., 2009; Fratta et al., 2011). Examples like MAGE family members and PRAME have been extensively investigated for their immunotherapeutic potential (Quintarelli et al., 2011; Thomas et al., 2018). Despite their promise, caution is warranted due to potential adverse effects, as seen in clinical trials targeting CTAs like MAGE A3, emphasizing the need for careful consideration in their application.

1.8.4 Tumor antigens as biomarkers-scopes and challenges

Tumor antigens hold significant promise as biomarkers for various aspects of cancer management. Elevated levels of tumor antigens in biological fluids or tissues can serve as indicators of cancer presence, facilitating early detection and diagnosis. Commonly used tumor antigen biomarkers include prostate-specific antigen for prostate cancer, carcinoembryonic antigen (CEA) for colorectal cancer, and alpha-fetoprotein for HCC. The expression profile of tumor antigens can provide valuable prognostic information regarding disease aggressiveness, metastatic potential, and patient survival outcomes. High levels of certain tumor antigens may correlate with advanced disease stage, tumor recurrence, or poor prognosis. Tumor antigen expression patterns can predict the response to specific therapeutic interventions, guiding treatment selection and optimization. For example, HER2 expression serves as a predictive biomarker for the response to HER2-targeted therapies in breast cancer. Changes in tumor antigen levels over time can serve as surrogate markers for monitoring treatment response, disease progression, and recurrence. Serial measurements of tumor antigen biomarkers, such as CA-125 in ovarian cancer, facilitate dynamic assessment of treatment efficacy and disease burden (Bai et al., 2020). Despite their potential clinical utility, tumor antigens pose several challenges and limitations as biomarkers for cancer. Many tumor antigens lack the requisite specificity and sensitivity for accurate cancer detection and monitoring. Cross-reactivity with normal tissues or low expression levels in early-stage disease can limit their diagnostic utility. Tumor antigen expression can exhibit significant heterogeneity among different cancer types and within individual tumors. Tumor heterogeneity poses challenges to the development of universal biomarkers and necessitates personalized approaches to biomarker selection and monitoring. Nonspecific elevation of tumor antigens may occur in benign conditions or other malignancies, leading to false-positive results and unnecessary diagnostic interventions. Conversely, certain cancer types may exhibit low or absent expression of commonly used tumor antigens, resulting in false-negative findings. The detection and quantification of tumor antigens rely on various laboratory techniques, each with its own inherent limitations. Assay standardization, variability in sample processing, and assay interference factors can introduce variability and impact result accuracy (Mehnert et al., 2017).

Table 1.2 Tumor antigen-based biomarkers and their potential clinical uses.

Tumor antigen type	Subtype of tumor antigen	Examples	Potential clinical uses	References
Tumor specific antigen	Neoantigens	Genomic neoantigen variants	Biomarkers for response to immunotherapy of lung cancer, glioblastoma, leukemia	Xie et al. (2023)
	Oncoviral antigens	DNA of EBV (Epstein-Bar Virus), HPV (Human-Papillomavirus) and MMTV (Mouse Mammary tumor virus) viruses	Biomarkers for breast cancer	Hammerl et al. (2018)
	Endogenous retroviral elements	HERV mRNA	Biomarker for early detection of breast cancer, melanoma	Curty et al. (2020)
Tumor associated antigen	Antigens against genes upregulated in tumors	ANXA5 (Annexin A5), FKBP10 (FKBP Prolyl isomerase 10), MSN (Moesin), PYGL (Glycogen Phosphorylase)	Biomarker for early detection of glioma	Zhong et al. (2021)
	Cancer germline antigen	MAGEB2 (MAGE Family member 2)	Biomarker for predicting response to immunotherapy in laryngeal cancer	Cui et al. (2022)
	Differentiation antigens	Core fucosylated prostate cancer antigen	Biomarker for differentiating different stages of prostate cancer	Lang et al. (2019)

Tumor antigens represent promising biomarkers for cancer detection, diagnosis, prognosis, and treatment response assessment. Despite inherent challenges and limitations, ongoing research endeavors continue to unravel the complexities of tumor antigen biology and refine their clinical utility. With further advancements in technology, multidisciplinary collaborations, and translational research efforts, tumor antigens hold the potential to revolutionize cancer diagnostics and therapeutics, ultimately improving patient outcomes and quality of life (Table 1.2).

1.9 Conclusion

Cancer can be of several types and the major types of cancer are lung cancer, liver cancer, prostate cancer, colorectal and breast cancer. A critical aspect of cancer detection, progression and response to therapy of cancer is the ability to track the various aspects using biomarkers. Different biomarkers are being currently used and many are under research for future uses. The major classes of biomarkers include mutant genes (germline and somatic mutations), epigenetic mutations, extra-cellular circulating nucleic acids. Some of the major genes that act as biomarkers are the mutated genes of EGFR, KRAS, TP53, SRC, PI3KCA, MYC, PTEN. In case of liver cancer, the major biomarkers include AFP, GPC3, Osteopontin, miR-21, p62/IMP2. Various tumor-specific antigens and tumor associated antigens are being increasingly looked upon as potential biomarkers across a variety of cancers. Further research into biomarkers will be highly interesting as it will be critical in the fight against cancers.

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CHAPTER

Understanding the role of biomarkers in cancer

2

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2.1 Introduction

The majority of patients with cancer receiving chemotherapy will experience severe toxicity because the response rates to single anticancer medication therapy are far lower than those for other diseases, and because anticancer drug doses that are successful frequently approach or surpass dangerous dose levels. Finding a patient group that will probably respond well to anticancer medication treatment is crucial. Many biomarkers have lately been investigated in an effort to overcome this problem. Furthermore, a large number of pharmaceutical companies have produced a large number of molecular-targeted medicines, some of which are now being used in clinical settings. One class of molecularly targeted agents works against tumor cells by altering a target specific to tumor cells. The creation of biomarkers is required to forecast how these drugs will affect the pertinent targets. The aim of biomarker development is to create methods for response rate, progression-free survival (PFS), and overall survival (OS) prediction in relation to molecularly targeted medicines. Biomarkers are thought to benefit patients and doctors alike if they enable us to identify a patient population that may respond well to treatment. Although no trustworthy biomarkers have yet been found for tumor-environment-specific molecular-targeted agents like antiangiogenic drugs, biomarkers are anticipated to reduce development costs and duration, as well as the number of patients enrolled in clinical studies, by providing useful information for new drug

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development. Recently, the advent of molecular-focused medicines with various targets has confounded the study of biomarkers. We will try to cover current developments and the practical implications of biomarker research in this overview.

2.2 Types of biomarkers in cancer

The role of biomarkers in cancer is growing, and more accessible molecular indicators that may be used for noninvasive monitoring of body fluids would be very beneficial to patients cancer. The term "biomarkers" refers to endogenous or injectable substances whose presence or metabolism is correlated with significant physiological processes associated with a disease or its effects. It would be helpful to describe or identify them as molecular entities so that they can be compared across platforms and labs. Modifications in the genetic, epigenetic, proteomic, glycomic, and metabolomic profiles of normal tissues, as well as modifications in imaging, can be indicative of cancer biomarkers. Every application for cancer biomarkers has unique goals, traits, and testing intervals.

2.3 Genetic biomarkers

The process of carcinogenesis involves the accumulation of genetic and epigenetic alterations that enable cells to evade the complex system of checks and balances that maintain the homeostatic equilibrium between cell division and apoptosis. According to Elenbaas et al. (2001), Weinberg and his colleagues' experimental work has demonstrated that the conversion of a primary cell into a malignant one in vitro necessitates changes in the functioning of a few mechanisms that control the growth, division, position, differentiation, and life span of cells. The idea that the morphological changes that accompany cancer progression could be determined through the gradual accumulation of genetic mutations gained popularity in the early 1990s due to studies conducted on cancer tissues.

The term "mutator phenotype" refers to the somatic mutations that develop and accumulate in cancer cells at a rate that is noticeably higher than in normal cells. According to Bielas et al. (2006), cancer cells' capacity to acquire mutations is essential for both the disease's progression and the quick emergence of resistance to cytotoxic cancer therapies. The mutator phenotype can arise from inherited genetic flaws that predispose individuals to particular cancers. These defects can be induced by a variety of mechanisms, including errors in DNA polymerase, apoptosis, specific DNA repair pathways, and cell-cycle regulatory defects. For instance, mutations in genes involved in the DNA mismatch repair mechanism are linked to microsatellite instability in patients with HNPCC (hereditary non-polyposis colorectal cancer). On the other hand, a significant portion of colorectal tumors displaying microsatellite instability were discovered

to have promoter hypermethylation silencing of these genes rather than mutations in the mismatch repair genes.

DNA structural changes ranging from small nucleotide sequence changes, like point mutations that impact a single nucleotide at a crucial position in a cancer-related gene, to chromosomal alterations involving millions of base pairs, such as translocations, deletions, or amplifications, are all considered mutations in cancer cells. These several types of changes frequently coexist in one tumor. The tumor suppressor TP53, which gets mutated or loses alleles in around half of human cancer cases, is a classic example of such a cancer gene.

Under specific stress conditions, such as DNA damage, the transcription factor TP53, which has 393 residues, controls the expression of several genes involved in anti-proliferative responses. Thus in genotoxic settings, this gene serves as a crucial defense against premature cell proliferation. The International Agency for Research on Cancer has a database called the TP53 (http://www.p53.iarc.fr/) that contains around 24,000 somatic TP53 mutations that have been found in nearly all types of human malignancies. The bulk of these mutations (74%) are missense substitutions, which are caused by single nucleotide substitutions that group together within exons 5–8. These substitutions change the protein domain's shape and/or biochemical activity, which is implicated in particular DNA binding. It was discovered in the early 1990s that TP53 mutations were happening in a non-random manner and that there were notable variations in the patterns of mutations between malignancies that are closely linked to response to environmental mutagens. Consequently, molecular epidemiology uses TP53 mutations as a biomarker primarily for their potential to report certain mutagenic exposures. It has been shown that there are notable variations in the mutation patterns of common malignancies when compared to geographic variations in the incidence; these variations may be due to varying exposure to different environmental carcinogens. The importance of TP53 mutation identification in bodily fluids in early cancer detection has been the subject of numerous investigations.

For instance, in patients with chronic obstructive pulmonary disease, mutations can occasionally be found in the DNA of exfoliated bronchial cells or sputum (Wang et al., 2006). Given that plasma contains minute amounts of free DNA fragments released by necrotic or apoptotic normal and cancer cells, it may be the most intriguing source of DNA for early cancer diagnosis. There have been reports of TP53 mutations in plasma DNA in patients with liver, lung, pancreatic, and colon malignancies. For instance, up to 5 years before liver cancer manifested itself, a particular TP53 mutation (at codon 249) produced by aflatoxin was found in the plasma of non-cancer patients who were chronic carriers of the Hepatitis B virus. We have demonstrated that the presence of KRAS and/or TP53 mutations in the plasma DNA of healthy individuals was predictive of bladder cancer risk in sizable prospective research (Gormally et al., 2007). However, it is uncertain if the DNA of mutant TP53 plasma cells comes from precancerous lesions or clinically undiagnosed cancer, or from normal cells exposed to mutagens.

2.4 Protein biomarkers

Enhancing the methods for protein biomarker identification has the most promise to enable biomarkers for cancer. Furthermore, although the technology for identifying these biomarkers is still in its infancy, there is interest in the possibility of using other compounds, like smaller molecules (metabolomics) and carbohydrates (glycomics), as biomarker diagnostics. Since each gene can produce tens, if not hundreds, of different species of protein through alternative splicing and over 100 distinct post-translational modifications, proteins are more diverse than DNA or RNA and so carry more information than nucleic acids. Furthermore, many physiologic alterations are not visible at the nucleic acid level because they are mediated post-transcriptionally. Moreover, proteins exhibit greater dynamism and reflect the physiology of cells. For instance, a single double-strand DNA break in a cell can quickly trigger a series of events known as protein phosphorylation (Aebersold et al., 2005).

2.5 Circulating biomarkers

Nucleic acids (cell-free DNA and RNA), circulating tumor cells, microvesicles distributed in the bloodstream, proteins and auto-antibodies, and circulating tumor cells are examples of circulating biomarkers that can be used to assess tumor load and metastatic potential in addition to offering a chance to look into specific molecular changes within a tumor. The current focus on so-called "liquid biopsies" is due to the fact that these biomarkers offer a potent substitute for performing invasive biopsies of particular organs for molecular research (Marrugo-Ramírez et al., 2018). Many articles have reported on a multitude of circulating biomarkers linked to cancer. A number of these biomarkers have proven to have clinically meaningful classification power in differentiating between benign controls and malignancies (differential diagnosis). For instance, the FDA has classified ROMA (CA125+HE4) and OVA1 (CA125, transthyretin, apolipoprotein A1, beta 2 microglobulin, and transferrin) as Class II devices. These are quantitative blood tests designed to identify patients with pelvic masses who might benefit from being referred to a gynecologic oncologist for their surgery. They do this by using particular algorithms to provide a risk score for the existence of ovarian cancer (Lokshin et al., 2021).

2.6 Imaging biomarker

Oncology makes extensive use of both imaging biomarkers (IBs) and biospecimen-derived biomarkers. Biomarkers are used in healthcare settings for disease screening, cancer diagnosis and staging, directing patient stratification, predicting

and tracking therapeutic efficacy and/or toxicity, and focusing on surgical and radiation therapies. According to Yap et al. (2010), biomarkers are used in research to direct the development of investigational drugs as they move along the pharmacological audit trail. They can show whether a drug targets, inhibits targets, modifies biochemical pathways or alters the pathophysiology in response to a drug, is effective in treating a particular patient group, or tracks drug resistance.

IBs regularly contribute to the field of cancer research. The Food and Drug Administration states that early regulatory approval of novel medications can result from the use of IBs to monitor patient response to treatment before a survival benefit is evident. By demonstrating receptor occupancy, for instance, IBs can demonstrate the existence of drug targets and target inhibition. When it comes to providing serial non-invasive mapping of the tumor state during treatment, IBs have a special potential. For instance, increases in the absolute values of the 18FFDG PET maximum standardized uptake value (SUVmax) early in therapy, or changes in this value at baseline, have been used to show nonspecific responses to treatment or as proof of mechanism in drug development. Pharmacodynamic (PD) alterations and treatment response have been assessed using dynamic contrast-enhanced ultrasonography area under the curve values and dynamic contrast-enhanced CT or MRI-derived Ktrans (Lassau et al., 2012; O'connor et al., 2012). The application of IBs has resulted in enhanced surgical and radiation dosage delivery margins (Taylor et al., 2014).

IBs possess four essential qualities. They are a subset of all biomarkers, to start. They can either be qualitative or quantitative. While additional measurements that do not come under this definition—such as the ACR BI-RADS category, clinical tumor, node, and metastasis (TNM) stage, or objective response—are categorical measurements and are also significant IBs, quantitative IBs—measured on an interval or ratio scale28—are employed in patient treatment.

2.7 Biomarker discovery, validation, and verification

The field of clinical studies has always depended heavily on the creation of protein biomarkers. The term "protein biomarkers" has been used in over 50,000 publications in PubMed since 2019. Biomarker discovery, verification, and validation comprise the three stages of the biomarker development pipeline (Bime et al., 2020).

Before beginning any kind of treatment, biomarker research for any illness, but especially cancer, needs to be carefully considered. The following procedures should be included in a biomarker discovery study: identifying the disease type, selecting patients based on factors like age, sex, and other characteristics, numbering patients and controls, etc. (Issaq & Veenstra, 2019). Optimizing the quantity of samples is necessary, as was previously stated. A minimum number of samples must be collected throughout the biomarker discovery phase of the

development process in order to meet statistical requirements. These samples must be sufficient to ensure accurate results free from high rates of false positives or false negatives. Nonetheless, the majority of patients or control samples result in issues with cost, efficiency, and ethics. Ideally, calculations based on prior information or statistical theory should be used to determine the ideal sample size. Power calculation is the standard method for determining the ideal sample size. Classification algorithms seek to maximize prediction accuracy; nevertheless, this approach has some issues that arise from the assumption of a high degree of correlation between data points and of maximizing the power to differentiate classes (de Valpine et al., 2009). Although many strategies have been put out, none of them have been able to fully get around all of the obstacles. The following provides an explanation of each biomarker discovery approach along with the recommended sample size for each stage of the process.

2.8 Biomarker discovery

In order to generate an initial list of proteins that might be implicated in the progression of the disease, the protein biomarker identification step entails measuring a large number of proteins in various samples. This process is mostly based on comprehensive, untargeted proteome research. In order to identify and quantify potential biomarkers, MS is essential. Any kind of specimen can be used in this phase, such as a cell line, mouse model, or a range of physiological specimens from humans, in order to create a binary comparison between healthy and diseased tissues (as the control), free from any "contamination" by other illnesses or other situations. One instance is the discovery of a protein that has a unique expression profile in patients with inflammatory bowel disease but not in healthy controls. Different samples of an illness can be utilized as a case or control when the goal is to identify a particular biomarker in a particular subtype of the disease. Usually, only a small sample size (10) is used in the discovery stage due to its low throughput, expense, and logistical challenges. A high false-positive rate may result from the small sample size and lack of analytical diversity. Verification and validation are therefore required after the identification of candidate proteins and are carried out using a larger number of samples. During the biomarker verification stage, many hundred samples are assessed in order to identify the extremely sensitive and specific biomarkers. Additionally, rather than measuring each peptide's relative quantification in this phase, the absolute amount is measured (Issaq & Veenstra, 2019).

2.9 Biomarker verification

Numerous samples are required to corroborate the potential biomarkers found during the discovery process, as was indicated in the preceding section. The relative concentrations of the putative biomarkers in a sufficient number of patient

specimens are determined during the verification step. In order to capture more variance in the population, the analysis is carried out in this phase on a bigger sample size consisting of a wider variety of patients and normal specimens. To achieve a statistically significant measure of the possible biomarker, the cohorts of specimens should be expanded to include samples from both healthy and disease-affected donors, in addition to those with similar diseases. For instance, a wide variety of ages of men and women, including pre- and postmenopausal women, should be represented in the samples. Creating a rapid, targeted test capable of analyzing hundreds or even thousands of samples and identifying as many candidates as feasible is the difficulty. This can be avoided by implementing a biomarker candidate verification step, which guarantees that the most likely biomarkers found during the initial stage are sent to the costly validation stage. As alternatives for biomarker verification, enzyme linked immunosorbent assay (ELISA), mitochondrial RNA modification (MRM) (Arora et al., 2019), premembrane protein (PRM) (Zhou et al., 2019), along with stable isotope-labeled internal standards, have been thoroughly investigated thus far (Issaq & Veenstra, 2019).

In order to identify and quantify biomolecules such as proteins, antibodies, glycoproteins, antigens, and hormones for biomarker verification based on the antibodies-antigens complex that yields measurable results, ELISA is a sensitive, high-throughput immunological technique. The four fundamental processes of an ELISA are coating (with an antigen or antibody), blocking (usually achieved by adding bovine serum albumin), detection, and readout. Step 3 involves adding a substrate that generates color, which results in detection. ELISA is divided into many types of assays, such as direct, indirect, sandwich, and competitive, based on the types of analytes and antibodies conjugated on the surface and their production signals. The ELISA immunoassay was created by Ourradi et al. (2017) to quantify quantitatively two novel biomarkers, C3f and V65, which appear to be specific to osteoarthritis and may be used to detect the illness early.

MRM, often referred to as selective reaction monitoring, or SRM, is crucial for research on human body fluid proteins and metabolism as well as pharmacological and drug analysis (Issaq & Veenstra, 2019). These instruments have the ability to detect certain fragment ions (MS2) based on m/z and then choose a parent peptide ion (MS1) of the target protein based on $/z$ to promote CID. The MRM method offers two benefits. First, a label-free operation without the requirement for antibodies and excellent sensitivity and selectivity reduces the preparative workflow. Second, throughput can be increased by quantifying many peptides at once. In a recent study, Chi et al. used SISCAPA-MRM and MRM to quantify 30 possible oral cancer biomarkers in saliva and plasma samples. These biomarkers were eventually found and/or confirmed (Chi et al., 2020). In a different study, Sjödin et al. (2017) assessed the ubiquitin concentration in cerebrospinal fluid as a biomarker for neurological dysfunctions using a method that measured ubiquitin concentration, specifically by coupling SPE and PRM–MS.

PRM is a very accurate and deterministic alternative approach for quantifying particular molecules. PRM provides parallel monitoring of each segment from the precursor ion and a full scan of any transfer by the ion. Because of this method's simplicity, it can be applied repeatedly to compounds that have already been characterized, like cancer biomarkers. For instance, Bao et al. (2022) used targeted PRM and MS-based quantitative proteomics to find and validate COPA as a potential predictive biomarker in freshly frozen cervical cancer tissue samples in 2022. Studies utilizing SRM and PRM have verified that for protein measurement, both focused techniques have comparable linearity, dynamic amplitude, accuracy, and repeatability with respect to relative sensitivity (Gallien et al., 2012).

2.10 Biomarker validation

In the validation phase, which follows the identification of a limited number of biomarkers during the verification phase, the external reproducibility is examined separately from the cohort. Compared to the discovery stage (e.g., < 100), the biomarker validation phase requires a larger number of clinical samples (e.g., > 1000) (Issaq & Veenstra, 2019). The most widely used method for validating biomarkers to date is ELISA, which has the capacity to concurrently predict many samples. Analytical validation and clinical validation are the two categories of validation.

2.10.1 Analytical validation

Analytical validation is the process of analyzing biomarker efficiency to verify that a test can be repeated and has sufficient sensitivity and specificity for the intended use. Clinical validation involves demonstrating the degree of connection, dependability, and clinical phenotype or desired outcome (Goossens et al., 2015). We will cover a number of recent analytical validation studies of cancer biomarkers that provide examples of this methodology.

In order to find 90 protein biomarker candidates and validate them using both MS and ELISA techniques, Park et al. (2017) adopted a proteomics strategy. Hurley et al. assessed serum samples from patients with high-grade ovarian cancer in 2020 and found five paraneoplastic antigens and three tumor-associated antigens. Two separate sample sets—validation I, including 164 samples, and validation II, comprising 150 samples—were used in the study's validation screening with ELISA and WB (Hurley et al., 2020). Zheng et al. reported on the use of DIA-MS-based technology in liquid biopsies in 2020. In the fluid biopsy of patients with colorectal cancer, they looked for EV protein/phosphoprotein indicators. They found FN1, S100A9, fibrinogen alpha chain (FGA), and HP, with notable protein phosphorylation and expression differences. According to their findings, FGA + crEV has a 65% sensitivity for early detection of adenoma patients and an almost

100% sensitivity for the diagnosis of colorectal cancer. The DIA-MS quantification of FGA and crEVs across three groups was ultimately verified by PRM-MS (Zheng et al., 2020). Table 2.1 lists the key steps and several techniques that are commonly employed in analytical validation.

2.10.2 Clinical validation

The propagation of a biomarker that has been identified as a viable candidate in the laboratory into a format that meets clinical utility presents a difficulty in the development of clinical assays. The final stage in the development of biomarkers is clinical validation, which requires proving clinical validity and clinical utility in addition to confirming the correlation between the biomarker and the endpoint of interest. In addition to taking longer than the analytical method, this method verifies the test result's dependability and degree of correlation with the clinical phenotype or intended outcome. When developing acceptance criteria during the clinical validation stage, cohort studies and the kind of biomarker employed in the unique cancer screening should be taken into account. The various steps of the biomarker pipeline may employ different mass spectrometric approaches, but in the traditional clinical environment, a straightforward, repeatable test that doesn't require highly specialized knowledge or equipment is desired (Parker & Borchers, 2014).

2.11 Clinical applications and challenges

2.11.1 Biomarkers testing in clinical practice

A cancer biomarker is a quantifiable and assessable tumor attribute or bodily reaction that may be objectively quantified in the presence of cancer. The primary focus of this process is to evaluate a change in the genetic makeup or a variation in the levels of protein expression . Personalized cancer therapy incorporates various types of biomarkers to enhance diagnosis, prognosis, prediction of treatment response, and evaluation of drug-patient interaction. These biomarkers include diagnostic biomarkers for identifying tumors in healthy individuals, prognostic biomarkers for assessing the natural progression of the disease, predictive biomarkers for anticipating response to specific therapies, pharmacokinetic, PD, and pharmacogenomic biomarkers for evaluating drug–patient interaction, and surrogate biomarkers that can be used as intermediate indicators to determine early response or resistance to treatment (Fig. 2.1).

The current consensus is that medicines that specifically target genetic abnormalities responsible for driving cancer genesis and progression can be effectively explored as rational treatment options . However, there is currently only a restricted range of successful targeted medicines available, which are linked to strong predictive biomarkers for treating individuals with solid tumors. The BCR-ABL fusion gene is one of the initial

Table 2.1 Techniques employed in analytical validation.

Phase	Techniques	Typical number of specimens	References
Biomarker discovery	10 specimens	• Mass spectroscopy-based gel electrophoresis; • Mass spectroscopy-based capillary electrophoresis-MS; • Mass spectroscopy-based gas chromatography-MS; • Mass spectroscopy-based liquid chromatography; • Offline NMR with liquid chromatography; • Enzyme-linked immunosorbent assay; • NMR with mass spectrometry; • Immunohistochemistry blotting	Zhang et al. (2019)
Verification	100 specimens	• Enzyme-linked immunosorbent assay; • Multiple reaction monitoring; • Parallel reaction monitoring; • Single reaction monitoring	
Validation	>100 specimens	• Enzyme-linked immunosorbent assay; • Multiple reaction monitoring; • Parallel reaction monitoring; • Surface plasmon resonance; • Western blotting	Park et al. (2017), Zheng et al. (2020), Del Campo et al. (2015), Van Steenoven et al. (2020)
Clinical validation	1000 specimens	• Enzyme-linked immunosorbent assay; • Enzyme-linked immunospot; • Flowcytometry	

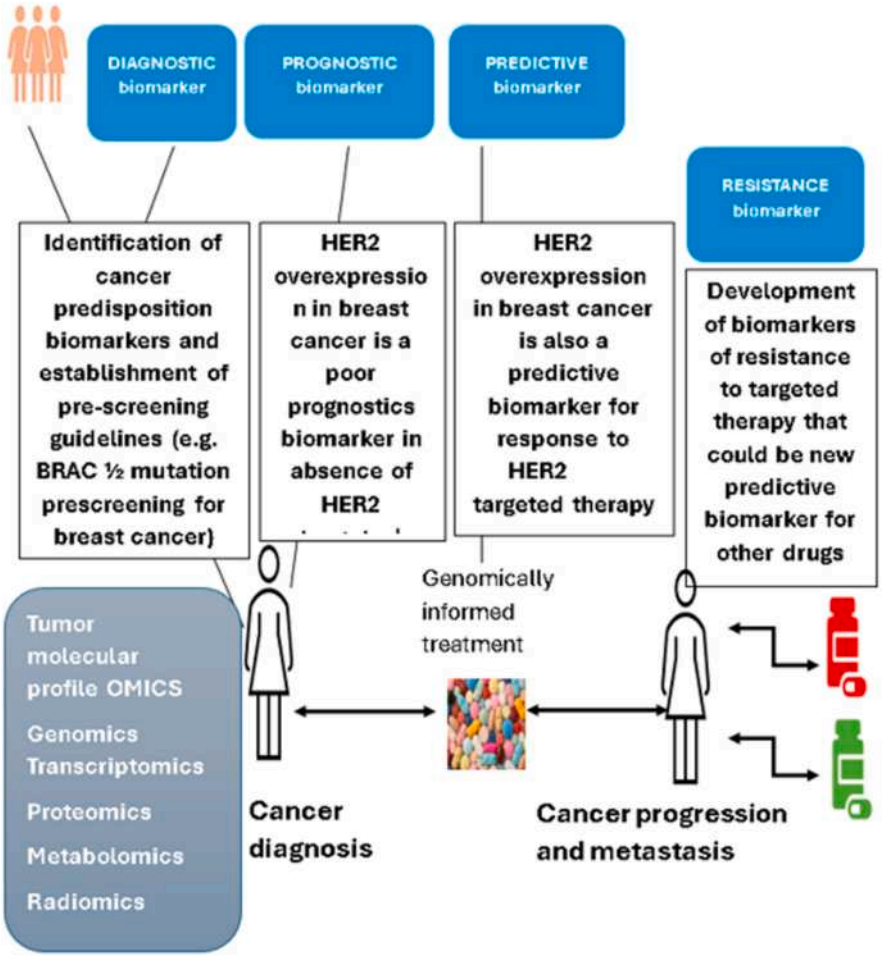

FIGURE 2.1

Types of biomarkers in the multistep drug development process.

predictive indicators identified for selecting treatment. The discovery of the translocation between chromosomes 9 and 22, also known as the Philadelphia chromosome, was made in the 1960s in patients with chronic myelogenous leukemia. It was later found that this translocation can be used to predict the response to imatinib, a drug that was approved by the Food and Drug Administration (FDA) for treating this condition in 2001 . The advancement of contemporary and intricate molecular and pharmacological technologies has subsequently led to a reduction in the duration required to progress from the identification of genomic alterations in drivers to the approval of drugs that are tailored to match them by the FDA.

Currently, there are ongoing clinical trials testing other potentially effective medications with reliable predictive biomarkers, such as Larotrectinib-nib. These trials specifically target malignancies that have NTRK gene fusions, regardless of the tumor's histology . Furthermore, significant progress has been made in the advancement of second- and third-generation drugs. These compounds enhance the effectiveness of current anticancer medicines by specifically addressing the mechanisms of resistance to first inhibitors. An illustrative instance is Osimertinib, a drug that specifically targets EGFR T790M mutations in non-small cell lung tumors. It has been shown to increase the PFS from 8.5 to 17.2 months in the initial stage of treatment, surpassing the efficacy of other conventional EGFR targeting tyrosine kinase inhibitors . Ceritinib has proven to be a successful biomarker-driven targeted therapy for ALK-altered nonsquamous non-small cell lung cancer. It has shown a considerable improvement in PFS, increasing from 8.1 to 16.6 months compared to standard chemotherapy . In order to further enhance the current state of affairs and expedite the process of developing drugs, it is imperative that research endeavors remain concentrated on identifying predictive biomarkers of response, as well as intermediate end points that can offer early indications of effectiveness or resistance to treatment . Before incorporating these biomarkers into regular clinical practice, it is necessary to subject the assays to preclinical validation using rigorous procedures, and then proceed with clinical qualification in prospective studies.

2.11.2 Limitations and pitfalls of biomarker utilization

One main reason is that most biomarkers are dealing with detecting diseases at an early stage in humans that have different ages, sex and ethnicity. Another important fact is to find a protein or a metabolite at an extremely low concentration level among thousands of other proteins and metabolites. To improve sensitivity and specificity, there are different strategies: potential solutions are listed as follows:

1. Improve the assay (e.g., antibody with a higher specificity and/or in combination with a detection conjugate with a higher sensitivity),
2. Combine several markers,
3. Check for subpopulations and stratify population (e.g., matched by gender, age, pathology).

The current procedure for the search of biomarkers deals with potential errors in the study design that can be avoided in future studies as explained in Fig. 2.2

2.11.3 Overcoming barriers to biomarkers implementation

The issue of implementation is becoming more widely acknowledged, and efforts to address the situation have been initiated. Funding agencies have recognized the significant financial requirement for supporting biomarker validation and

FIGURE 2.2

Pitfalls and failures in biomarker identification.

qualification studies. Notably, recent calls for proposals for collaborative projects under the EU FP7 program have highlighted this need (http://ec.europa.eu/research/participants/portal/page/fp7_calls). The number 30 is enclosed in brackets [30]. Other instances include the Joint Programming Initiative in Neurodegenerative Diseases, which is providing funding for the initial trial call for research projects in the "Optimization of biomarkers and harmonization of their use between clinical centers" field; and the recently established ERA-Net TRANSCAN, which has put forward the subject of "Validation of biomarkers for personalized cancer medicine".

In the example of diabetic nephropathy mentioned earlier, a clinical trial called PRIORITY (FP7 2012–2016) is being initiated to investigate the potential advantages of intervening in early diabetic kidney injury. This intervention is based on a set of urinary protein biomarkers. Additionally, the "Early Prevention of Diabetes Complications in Europe" (e-PREDICE) project has received funding to study changes in biomarkers related to microvascular damage, endothelial function, oxidation, and inflammation. These changes are expected to occur as a result of different drug treatments aimed at preventing diabetic complications at an early stage. Similar initiatives are also in progress in various other domains and nations, such as the United States, where the National Cancer Institute's Early Detection Research Network (http://edrn.nci.nih.gov) is actively involved. These advancements represent significant progress, as they move the focus from discovering biomarkers to applying them in clinical settings. However, the whole implementation process still seems to be significantly lacking in funding.

The European Medical Research Council (EMRC) has recently published the ESF/EMRC "ahead look" (http://www.esf.org/emrc; May 2011) to help improve the implementation of research findings in medical practice. This publication discusses the various challenges that need to be overcome in this process. In order to lead the development of a plan for implementation and determine the specific steps that need to be taken for proteomics findings, the European Kidney and Urine proteomics COST Action held a session on the clinical application of research findings related to kidney diseases during its regular meeting in Madrid in 2011. The symposium aimed to gather perspectives on the implementation process from researchers, doctors, representatives from biobanks, industry, funding organizations, and regulatory bodies. Collectively, these initiatives demonstrated the importance of implementing a more structured approach to biomarker research, which should consider the requirements and viewpoints of all stakeholders involved, ranging from scientists in the research laboratory to end-users such as patients, physicians, and regulatory bodies. To streamline the adoption of clinical proteomics findings (Fig. 2.2), we recommend the following steps:

- Conduct preliminary investigation and verification for the particular context in which it will be used. If the result is positive.
- Seek the expertize of a suitable interdisciplinary panel (explained in detail below) to assess the evidence and, if favorable, offer recommendations for future study design;
- Seek funding and simultaneously seek samples from biobanks, if they are accessible, or commence new sample collection based on the panel's suggestions;
- Conduct biomarker assessment; • Consult the panel for appraisal of the supplementary data and, if favorable, for advice on designing clinical studies; • Submit a grant application and conduct an intervention study to assess the anticipated advantages. It is advisable to evaluate hard endpoints, if they can be achieved within a realistic timeframe. If it is not feasible, and the biomarkers are

deemed to have potentially life-saving clinical value, validated surrogate endpoints may be utilized, requiring further evaluation to determine the definitive outcomes. • Request an appraisal of the evidence from the intervention study from the panel. If the result is positive; • Integrate into clinical practice, sometimes with restrictions, until sufficient data on definitive outcomes is available;

- Utilize feedback systems to assess the cost-effectiveness, clinical adoption, challenges in normal implementation, and unforeseen collateral issues.

2.11.4 Future directions and emerging technologies

In recent decades, there has been a significant increase in the identification of biomarkers. In several domains of clinical medicine, a multitude of potential indicators have been suggested using "hypothesis-driven" methods. The advancement of high throughput technology has significantly increased the speed of discovery, leading to a large number of potential markers. Regrettably, both the scientific community and the health care environment lack the required capacity to effectively evaluate a significant number of putative biomarkers in large patient groups . Therefore the implementation of bio markers in clinical practice is hindered by the substantial expenses associated with biomarker validation and the lack of clear prioritization criteria. The selection of markers to validate is frequently influenced by commercial factors, irrespective of the appeal of the underlying notion. The citation refers to a source authored by Rifai and colleagues in 2006.

Establishing robust collaboration relationships among academics, clinicians, and industry is crucial for the discovery and application of groundbreaking biomarkers in clinical settings. The "omics" revolution can provide us numerous promising candidates, but it cannot direct us toward their execution. Furthermore, it is necessary to investigate and create connections between the alleged biomarker and the underlying pathological mechanisms of the disease of interest, in addition to implementing fast, efficient, and methodical methods for clinical validation. Biomarkers are most helpful when they offer insights into the activation of certain pathways, so acting as portals into the molecular foundation of the disease.

Given the intricate nature of typical medical illnesses, it is evident that individual biomarker tests are improbable to bring about revolutionary changes in clinical application. In the future, the use of multimarker techniques that evaluate various key components of the disease's pathophysiology may equip doctors with the necessary knowledge for diagnosing, prognosticating, and treating the condition. For instance, the future advancement of multimarker panels that are relevant to the pathophysiology of acute myocardial infarction (MI) could potentially revolutionize the clinical assessment and management of individuals with this condition. Although the population suffering from an acute MI is diverse and the disease's pathogenesis is complex, the current treatment is uniform. The treatment strategy is primarily modified depending on clinical examination, routine

laboratory testing, and the utilization of imaging methods to evaluate heart function and ischemia. Nevertheless, these indications are inadequate for forecasting the occurrence of ventricular remodeling and heart failure in patients experiencing an acute myocardial infarction.

In a pathophysiologically oriented biomarker-guided approach, clinical decision making would be guided by multimarker panels that provide information on various pathological processes, such as cardiomyocyte necrosis, the presence of ischemia, plaque vulnerability, pro-arrhythmic tendencies, the reparative response, matrix deposition, and metabolism. The selection of biomarkers would be guided by pathophysiological insights obtained from fundamental research. Implementing such a strategy would enable the clinician to differentiate patients who are at higher risk of developing dilative remodeling as a result of impaired resolution of postinfarction inflammation and overactive matrix-degrading pathways from those who are more likely to exhibit dominant pro-fibrotic responses, which are likely to lead to significant diastolic dysfunction. The number is 46. The development and implementation of such ambitious endeavors will inevitably encounter several obstacles but have the potential to ultimately achieve the visionary objective of personalized medicine.

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CHAPTER

Tools and technologies for biomarker discovery

3

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3.1 Introduction

Biomarkers are measurable indicators of biological procedures, conditions, or diseases. According to the National Institutes of Health definition, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Califf, 2018). They could be molecules in the blood, other body fluids, or tissues with defined structural, physiological, or anatomical characteristics. The biomarkers aid in disease diagnosis, identifying disease progress upon treatment, developing personalized medicines, and even identifying individuals with a potential risk of diseases (Aronson & Ferner, 2017). Depending on their applicability, biomarkers can be classified into five major classes: diagnostic biomarkers, antecedent biomarkers, screening biomarkers, staging biomarkers, and prognostic biomarkers. Diagnostic biomarkers are used to diagnose disease conditions. Troponin for myocardial infarction, catestatin for psychological stress response, and glutamate for visceral obesity are some examples of diagnostic biomarkers. Altered expression of these biomarkers can be used to diagnose their respective diseases (Bodaghi et al., 2023). Antecedent biomarkers or risk biomarkers indicate the risk of developing a disease. These biomarkers will be present before the onset of the disease. Patients with altered *APOE* (apolipoprotein E) gene are an indication of the pathogenesis of Alzheimer disease (Mayeux, 2004). Screening biomarkers detect subclinical forms of diseases. Early detection through screening biomarkers can lead to timely intervention, improving prognosis and survival rates. Staging biomarkers categorize different stages of the disease by assessing the severity. Accurate staging is crucial for selecting appropriate treatment strategies, predicting outcomes, and monitoring disease progression. Prognostic biomarkers predict the likely course of a disease, including disease recurrence, progression, and patient survival. These biomarkers provide insight into a disease's likely occurrence or recurrence by screening, monitoring, and assessing the levels of internal precursors (Bodaghi et al., 2023; Majkić-Singh, 2011).

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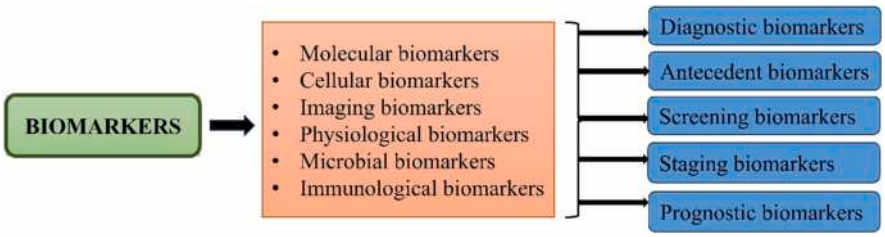

FIGURE 3.1

Different forms of biomarkers and their medical applications.

Biomarkers can exist in several forms, with different roles in detecting, diagnosing, and managing a disease (Fig. 3.1). Molecular biomarkers comprise nucleic acids to proteins of varying sizes as biomarkers and include chemicals, proteins, or genes (Laterza et al., 2007). Cells can be used as biomarkers and are considered as cellular biomarkers. The cell counts in blood, or the change in expression of cell surface markers can be included in the cellular biomarker category (Bodaghi et al., 2023). Imaging biomarkers are another category used as biomarkers. This is the most widely used class of biomarkers due to their availability, cost-effectiveness, and noninvasiveness. It comprises radiographic imaging, like computed tomography and (magnetic resonance imaging) MRI scans, visually representing the imaging cells, tissues, or organs. Functional imaging tools like positron emission tomography (PET) that can access metabolic processes also fall into this category (Garner et al., 2019). Another biomarker category, the physiological biomarkers, details the molecules associated with various conditions, such as stress. Studies have characterized stress-induced biomarkers, including cortisol, catecholamines, glucose, etc. These biomarkers can predict the onset of various stress-induced diseases (Noushad et al., 2021). Biomarkers are identified upon microbial infection and are utilized to identify the type of microbial infection. They are called microbial biomarkers. It includes the presence of microbial genetic material or microbial metabolites in the body and is used for disease diagnosis and prognosis (Gao et al., 2024). Several immune-related molecules, called immunological biomarkers, can also be considered biomarkers for identifying infections. The presence of antibodies against specific antigens and levels of immune response activating cytokines in the body fluids indicate the presence of infection (Orbe & Benros, 2023). These different forms of biomarkers provide evidence across various medical applications, including screening, diagnosis, prognosis, and treatment monitoring.

Biomarkers are crucial in understanding, diagnosing, and treating various diseases. Various biomarkers are determined based on the abnormal expression of specific molecules under specific disease conditions. Cardiovascular disease-associated biomarkers aid in the early detection and diagnosis of various heart

problems, increasing patient survival rates. Cardiac troponins, exosomal cargo, and cytokines associated with cardiac inflammation are biomarkers for diagnosing and prognosis cardiovascular diseases (Kim et al., 2023). Identification of biomarkers associated with diabetes helped patients identify prediabetic conditions, which, upon proper control, can reduce the early onset of diabetes. Hemoglobin A1c (HbA1c), fructosamine, glycated albumin, etc., serve as alternate biomarkers for diabetes (Dorcely et al., 2017). Neurogenerative disease biomarkers are identified and continue to expand for diagnosing and rendering neurological diseases. Micro RNAs (miRNA), long noncoding RNAs (lncRNAs), protein biomarkers, etc., serve as essential biomarkers for neurogenerative disease (Selvam & Ayyavoo, 2024). Novel biomarkers have been identified for detecting autoimmune diseases. Autoantibodies, anti-nuclear antibodies, anti-dsDNA, antiphospholipid, etc., are currently used biomarkers for identifying systemic chronic inflammation (Fenton & Pedersen, 2023).

Cancer biomarkers are molecules expressed abnormally as the cancer develops and progresses (Sarhadi & Armengol, 2022). High heterogenicity of cancer cells, development of drug resistance, difficulty in the early detection of cancer, and reduced treatment advancements in selective cancer targeting are the major complications in cancer diagnosis and treatment (Pucci et al., 2019). Advanced approaches to early detection and efficient methods for cancer treatment are necessary for improving the survival rate of patients. Biomarker discovery is paving a vital path in controlling and managing cancer at various stages of cancer progression.

The role of biomarkers in cancer detection is extensively studied and is applied to improve treatment outcomes and survival rates significantly (Das et al., 2023). The application of biomarkers in early cancer diagnosis and treatment is a vital area of research as they provide valuable insights into the early detection, treatment progression, and prediction of the risk of developing cancer. Early detection by biomarkers minimizes the involvement of other complex and expensive treatments required in the advanced stages of cancer. It can increase the survival rate of many cancers. The drastic change in proteome expression profile occurs when healthy cells transform into cancer cells. These serve as early stage biomarkers in cancer detection (Krishna Prasanth et al., 2023). These early biomarkers are used for prognosis purposes. Circulating tumor DNAs can be used as a prognostic marker for the early stage detection of various cancers. Altered human chorionic gonadotropin and deregulated specific miRNA levels can be used as prognostic markers in the development of ovarian cancer (Matsas et al., 2023). They are used to predict the efficiency of drugs against cancer. It is reported that codon-specific *KRAS* mutation can be used as a biomarker for determining the efficiency of trifluridine/tipiracil chemotherapy in colorectal cancer (van de Haar et al., 2023). They are even used for targeted therapy in cancer. Patients HER2-positive breast cancer are treated with trastuzumab and pertuzumab, and they can also be used for the treatment of other HER-2-expressing cancers like colorectal and non-small-cell lung (Oh & Bang, 2020). Breast cancer biomarkers *BRCA1* and *BRCA2* gene

mutations are used for identifying the potential risk of developing breast cancer in women (Metcalfe et al., 2010). Thus integrating biomarkers into cancer treatment allows for a highly targeted and effective practice that brings better outcomes to patients and cancer research. It offers early detection, accurate diagnosis, prognosis, and monitoring of cancer with high accuracy and efficiency. With a further advanced technological understanding, the role of biomarkers will further expand toward more precise or personalized strategies in the fight against cancer.

3.2 Applicable tools and databases used in biomarker discovery

Biomarker identification has evolved into an essential part of biomedical research and personalized medicine to improve health conditions, disease diagnosis, prognosis, and treatment. One of the most frequent strategies in this area is using high-throughput screening technologies, which create massive amounts of biological data. These data include a wide spectrum of biological molecules, such as genes, proteins, and metabolites. Analyzing these data involves using advanced bioinformatics tools that can manage, process, and interpret enormous amounts of data efficiently (Cui et al., 2022). The initial phase in biomarker identification is often to collect relevant biological data from public sources or experimental outcomes. This information contains genomic sequences, transcriptome profiles, proteomic patterns, and metabolomic markers. To find patterns and correlations that indicate possible biomarkers, such multi-omics datasets must be analyzed comprehensively using a combination of statistical methods and machine learning algorithms. These approaches help not only identify differentially expressed genes or proteins but also understand the underlying biological pathways and networks involved in disease processes (Waury et al., 2022). However, the identified candidates go through a number of validation stages. This involves utilizing multiple computational models to estimate the biological relevance and possible clinical utility of biomarkers. These models utilize current biological data, including approaches such as pathway analysis, network analysis, and molecular docking simulations (Nakayasu et al., 2021; Srinivasan et al., 2020). Incorporating clinical data, such as patient data, disease phenotypes, and treatment outcomes, refines the biomarker candidates, assuring their relevance in the clinical environment (Marcos-Zambrano et al., 2021). Furthermore, numerous computational can be utilized to explore the features of biomarkers, such as sequence analysis, structure prediction, and epitope mapping, to aid in assay creation. Also, advances in computer tools and algorithm development have permitted the deployment of more advanced methodologies, such as deep learning and artificial intelligence, which speed biomarker identification in many complicated disorders (Elbadawi et al., 2021; Xiao et al., 2021). To accelerate biomarker discovery, various specialized software, tools, and databases are

Table 3.1 List of applicable tools and databases used for the discovery of biomarkers.

Sl. No.	Name	URL/Github	References
1.	MetaFS	https://idrblab.org/metafs/	Tang et al. (2021)
2.	DeepKEGG	https://github.com/lanbiolab/DeepKEGG	Lan et al. (2024)
3.	GutBalance	http://39.100.246.211:8051/balance/	Yang et al. (2021)
4.	ExoBCD	https://exobcd.liumwei.org/	Wang et al. (2021)
5.	TPD (tipping point detector)	http://www.rpcomputationalbiology.cn/TPD	Chen et al. (2022)
6.	DESeq2	https://bioconductor.org/packages/release/bioc/html/DESeq2.html	Love et al. (2014)
7.	CyNetSVM	https://apps.cytoscape.org/apps/cynetsvm	Shi et al. (2017)
8.	MiRNA-BD	http://www.sysbio.org.cn/mirna-bd/	Lin et al. (2018)
9.	omniBiomarker	https://openebench.bsc.es/tool/omnibiomarker	Phan et al. (2013)
10.	C-Biomarker.net	https://github.com/trantd/C-Biomarker.net	Tran et al. (no date)

available that provide easy, visual tools for examining omics data, prioritizing biomarker candidates based on biological features, and comparing expression patterns across different situations. So, for further understanding, the names of useful tools and databases are listed in Table 3.1.

3.2.1 MetaFS

It is an online platform that provides the evaluation study of feature selection methods for biomarker discovery in metaproteomics. It integrates 13 different feature selection methods and assesses their performance based on four independent criteria: stability, relevance, redundancy, and classification accuracy. The tool allows users to upload their metaproteomic datasets. It automatically evaluates the performance of the feature selection methods, helping to identify the most well-performing methods for the specific data. also, by identifying the top-performing FS methods, MetaFS can assist the most promising biomarker candidates from the metaproteomic data (Tang et al., 2021).

3.2.2 DeepKEGG

DeepKEGG is a multi-omics data integration framework that uses deep learning to predict cancer recurrence and identify biomarkers. It constructs relationship matrices between genes, miRNAs, and pathways to enable local connectivity and model interpretability. DeepKEGG uses an attribution-based interpretability method to calculate the importance scores of genes and miRNAs to identify key biomarkers (Lan et al., 2024).

3.2.3 GutBalance

It is a web server for biomarker and disease prediction related to the human gut microbiome. It addresses the compositional nature of microbiome data, which is often neglected in supervised learning tasks. It also applies statistical learning to identify the microbial biomarkers from various molecular species (Yang et al., 2021).

3.2.4 ExoBCD

It is a comprehensive database that was constructed with the combination of the four high-throughput datasets, which provide a novel resource for biomarkers in the treatment and diagnosis of breast cancer. It provides a visualized and systematic analysis of the gene ontology and KEGG pathway analysis. Additionally, it also identifies the relationship between mRNAs and miRNAs, which is a valuable data source for the identification of the biomarker of breast cancer (Wang, Chai et al., 2021).

3.2.5 TPD

it is a web tool for detecting tipping points during the dynamic process of biological systems based on dynamic network biomarkers. It allows the identification of critical transitions in complex biological systems, such as disease progression or treatment. It is particularly used to assess drug mechanisms and action to optimize drug dosing. It also generates the dynamic network biomarker scores that may help to identify the differentially expressed genes (Chen et al., 2022).

3.2.6 DESeq2

It is a tool for identifying differentially expressed genes and potential biomarkers from the RNA-seq data. It uses statistical models to detect the genes in two or more experimental conditions. However, it also provides statistical significance measures like *P*-value or false discovery rates to prioritize the most promising biomarker (Love et al., 2014).

3.2.7 CyNetSVM

It is a user-friendly graphical interface that has been used to analyze breast cancer data to identify network genes and signaling pathways that are associated with cancer progression and also show the effectiveness in cancer biomarker identification. It used the NetSVM computational method to predict network biomarkers by integrating gene expression data and protein-protein interaction data (Shi et al., 2017).

3.2.8 MiRNA-BD

It is evidence-based bioinformatics software designed for microRNA biomarker discovery. It aims to identify the microRNA that can serve as biomarkers for disease diagnosis prognosis. It also searches for miRNA–mRNA networks and considers the independent regulatory power of miRNAs (Lin et al., 2018).

3.2.9 omniBiomarker

it is a web-based application that uses the NCI Cancer Gene Index data to guide the selection of biologically relevant algorithms for identifying biomarkers. It uses feature selection algorithms that analyze the curated cancer biomarkers and improve microarray-based clinical prediction performance (Phan et al., 2013).

3.2.10 C-Biomarker.net

It is a Cytoscape app that identifies cancer biomarker genes from various biomolecular networks. It also uses the data from the NCI Cancer Geene Index to guide the selection of biologically relevant algorithms for identifying biomarkers that can improve the performance of microarray-based clinical prediction (Tran et al., no date).

3.2.10.1 Overview of technologies for biomarkers discovery

The identification and discovery of biomarkers have become essential in many scientific domains, particularly drug development and research. Biomarkers are crucial for understanding the biological basis of diseases, developing targeted medicines, and providing personalized treatment. Biomarker discovery methods have advanced dramatically, transforming the approach to illness diagnosis, prognosis, and treatment. Biomarker discovery relies heavily on genetics. One of the principal methodologies utilized in this sector is the analysis of DNA modifications, such as single nucleotide polymorphisms (SNPs), as well as chromosomal abnormalities, including DNA rearrangements and variations in DNA numbers. Common approaches for genetic biomarker development include association studies and linkage analysis, which look for genetic changes that are

associated with diseases, environmental effects, and treatment responses. These genetic biomarkers are frequently linked to the etiology of diseases and are essential in establishing the relationship between genetic variants and disease or drug responses (Arbitrio et al., 2021; Dar et al., 2023). Furthermore, analyzing genomes from vast populations or specialized patient groups, it might identify genetic changes linked to certain diseases or conditions. These changes, commonly known as genetic markers or biomarkers, serve as predictors of disease risk, severity, or response to drugs (Dar et al., 2023). Traditional Sanger sequencing has been improved and, in some cases, prevailed by next-generation sequencing (NGS) methods including as pyrosequencing, sequencing by hybridization, sequencing by ligation, single-cell sequencing, and many more (Qi et al., 2020). The evolution of single-cell analysis improved biomarker identification by capturing cell heterogeneity, which plays an essential role in complicated diseases such as cancer. Single-cell RNA sequencing techniques identified various cell types and transcriptional patterns, revealing unusual biomarkers that were previously unknown in bulk analysis. However, breakthroughs like Roche have substantially boosted the throughput and efficiency of pyrosequencing for biomarker discovery (Lei et al., 2021). Furthermore, whole-genome sequencing is becoming more common, with platforms such as Illumina and Affymetrix dominating the market. These high-throughput tools have transformed the field, enabled whole-genome association studies, and moved us closer to personalized treatment. One of the primary benefits of whole-genome genotyping is its capacity to discover connections between genetic markers and complex traits or disorders through large-scale association studies (Agapito et al., 2020; Höglund et al., 2019). Genome-wide association studies (GWAS) are a common use of whole-genome genotyping, in which researchers assess the frequency of genetic variants in individuals with and without a specific disease or trait. By finding SNPs that are considerably more common in individuals with the disease, GWAS might pinpoint regions of the genome that may contain genetic risk factors or biomarkers relevant to the condition under research (Höglund et al., 2019). In addition, functional genomics is an evolving field that contributes significantly to biomarker development by revealing how genetic information is translated into functional biological processes and phenotypic features. Functional genomics is fundamentally concerned with understanding the function and regulation of genes, their interactions within cellular networks, and their impact on physiological and pathological situations (Nguyen & Caldas, 2021). Microarrays allow for the simultaneous monitoring of thousands of genes, giving an in-depth understanding of gene expression under varied situations. Transcriptomics, a critical component of functional genomics, involves profiling RNA transcripts to determine which genes are actively transcribed and in what quantities. RNA sequencing (RNA-seq) enables researchers to quantify gene expression in a high-throughput way, providing an overview of the transcriptome in both healthy and diseased states. Researchers can uncover dysregulated genes that serve as possible signs of disease presence or development by comparing transcriptome profiles of healthy individuals to patients

with certain diseases. Two basic forms of DNA microarrays are two basic forms of DNA microarrays that are cDNA microarrays and oligonucleotide microarrays. Platforms like Affymetrix and Illumina have set industry standards for reliability, accuracy, and consistency, which enable enormous genomic database development and robust gene expression research (Sánchez-Baizán et al., 2022; Wang et al., 2014). In addition to transcriptomics, epigenetics has gained attraction in biomarker development. Epigenomics is the study of heritable changes in gene expression that are not associated with changes in the DNA sequence and histone protein changes that influence gene expression while leaving the underlying DNA sequence unchanged. Epigenetic changes, such as DNA methylation and histone acetylation, are crucial for gene control and cellular identity. Dysregulation of epigenetic markers is linked to a variety of illnesses, including cancer and neurological problems. In biomarker development, epigenomic profiling gives valuable information on epigenetic fingerprints that can distinguish between health and disease states, suggesting possible biomarkers for early identification or monitoring of disease progression (Kumaraswamy et al., 2021; Singh et al., 2022). Furthermore, proteomics became popular as a biomarker discovery tool, due to its capacity to provide comprehensive insights into the proteome. Proteomics technology has improved significantly, with mass spectrometry (MS), protein microarrays, and bioinformatics tools all adding uniquely to the biomarker discovery pipeline. MS, label-free quantification approaches like as spectrum counting and intensity-based absolute quantification, and tandem mass tags are examples of instruments that improve the accuracy and reliability of biomarker discovery. Furthermore, fluorescence 2D difference gel electrophoresis (DIGE) and molecular imaging of protein spectra with MS directly on tissue slices are useful advances in proteomics. These methods improve the sensitivity and specificity of protein detection, allowing for more thorough protein characterization and localization inside tissues. Although fluorescent 2D DIGE and molecular imaging of protein spectra utilizing MS directly on tissue slices represent important advances in proteomics (Ura et al., 2021; Zhang et al., 2022). These methods improve the sensitivity and specificity of protein detection, allowing for more thorough protein characterization and localization inside tissues. Furthermore, protein microarrays have evolved as a high-throughput approach to focused proteomics. There are three primary types available: traditional protein microarrays produced of purified recombinant proteins, antibody microarrays, and reverse protein microarrays made from cell lysates. Despite limitations in specificity, sensitivity, and standardization, protein microarrays are a potential tool for parallel investigation of many proteins, which can help with biomarker development in a variety of disorders. Protein microarrays have evolved as a high-throughput approach in focused proteomics. Despite limitations associated with specificity, sensitivity, and standardization, protein microarrays offer a potential tool for the parallel study of numerous proteins, helping to biomarker identification in various disorders (Kwon et al., 2021; Li et al., 2021). However, peptidomics and metabolomics, which focus on identifying peptide fragments and endogenous

metabolites in biofluids and tissues, have proven useful in monitoring drug efficacy and safety, as well as identifying disease biomarkers. Recent advances in MS-based metabolomics include focused direct quantification of metabolites using standards and chip-based nanoelectrospray, which enhances sensitivity and reduces the impact of the matrix (Aderemi et al., 2021; Foreman et al., 2021; Yu et al., 2021). Despite these advances, metabolomics still faces obstacles such as a lack of complete metabolite databases, nonautomated structure assignments, and data analysis challenges. In conclusion, the discovery of biomarkers across various scientific fields has been revolutionized by advancements in genetics, genomics, proteomics, peptidomics, and metabolomics (Tolstikov et al., 2020; Zhang, Li, et al., 2021). These technologies have increased the accuracy, sensitivity, and throughput of biomarker identification, paving the path for more personalized and focused approaches to treatment. However, issues with data analysis, standardization, and cost remain, demanding ongoing innovation and collaboration within the scientific community to fully realize the potential of biomarker discovery.

3.2.10.2 *Case study*

Several studies were successfully designed to explore the complex biological mechanism and the responsible genes involved in the progression and found favored results that are helpful for a better tomorrow. This section will help (Table 3.2) understand how the researchers are involved and use different tools, techniques, applications, and algorithms to understand the complex biological mechanism considering the biomarker role.

At present several cancer infections are ongoing, which generate severe complications in the infected patient, and among them, breast cancer is one of the most vital ones. Keeping this as a concern, a study performed by Wang et al. targeted CXCL1–2 via the bioinformatics approach along with experimental validation to understand its prognostic value in breast cancer infection. Initially, a set of datasets from the three different databases were collected and analyzed such as ONCOMINE, GEPIA2, UALCAN, and cBioPortal, and for the expression

Table 3.2 List of selected successfully designed studies.

Sl. No	Target	References
1.	Breast cancer	Wang et al. (2021)
2.	Breast cancer	Pattar et al. (2020)
3.	Breast cancer	Jha et al. (2022)
4.	Lung cancer	Chen and Dhahbi (2021)
5.	Gastric cancer	Zhang, Xue, et al. (2021)

analysis, survival rate, and the mutation implication within it. Further, the network of the collected and analyzed data was generated and designed considering various parameters and further used to explore the biological activity followed by the associated pathways. Additionally, the possible drugs of the biomarker were identified from different databases, and further, a step of precise experimental investigation was performed, followed by statistical analysis. The overall study and investigation revealed that CXCL1–2 is associated with the poor progression of breast cancer, and via docking and experimental validation, Quercetin was found as a potential inhibitor, and the authors suggested that the obtained results required in vitro and in vivo validation (Wang, Yuan et al., 2021).

Similarly, another study by Patter et al. targets closely associated targets to understand the molecular activity of Coumarin-carbonodithioate derivatives followed by docking and energy calculation. In this study, 18 potential targets were used and prepared via the protein preparation wizard. Further, the total 14 derivatives were prepared via the maestro software, the docking analysis was performed, and the MM-GBSA of the complex was calculated. Based on subsequent steps, the overall study revealed that these derivatives can be potential inhibitors as they are successfully bound and inhibit the target (Pattar et al., 2020).

A study performed by Jha et al. used a computational approach to screen out the potential anti-Brest cancer inhibitor via the docking analysis followed by drug-likeness properties investigation. In this study, six most promising targets that play an essential role in the infection were used, and their three-dimensional structure was collected from the PDB database using their respective IDs. These collected proteins were prepared, and similarly, a phytochemical having an anticancer activity-based ligand was collected, and their library was designed and prepared. Furthermore, to use the potential one, the pre-drug-likeness investigation was performed. Finally, the docking analysis was performed using the set of collected ligands and the selected target structure to uncover the molecular activity mechanism via their interaction. The overall study shows that several compounds are effective towards the target. Among them, four compounds, along with their derivatives as the most promising ones, their favored drug-like properties and no-toxic activity suggest that these can be useful for the potential drug design (Jha et al., 2022).

As similar, another fatal cancer is lung cancer, which is harmful and complicated. To overcome and get a better understanding, another study was performed by Chen et al., who employed the fusion of a machine learning model to screen out the overlapped gene from lung adenocarcinoma and lung squamous cell carcinoma and validated via the random forest. In this study, the associated genes were collected from the TCGA website. Based on the set of algorithms, the genes followed by three or more algorithms were selected and considered biomarkers, and the ROC curve was analyzed. Further, based on the top gene selection and followed by their validation, a total of 17 biomarkers were selected and subjected to the ROC analysis, which revealed the gene's function, such as whether the genes are

upregulated or downregulated. Moreover, the enrichment analysis and pathways analysis were also done to understand the selected gene and their responsible related pathways which are helpful to understand the complete mechanisms. The overall study shows that the designed protocol has better effectiveness and identified biomarkers have a significant role in the prognostic potential (Chen & Dhahbi, 2021).

Among other cancers, gastric cancer is also generating a diverse complication in the host. Gastric cancer has a high mortality rate also. Therefore another study was designed by Zhang et al., which employed an advanced transcriptomics approach to identify the potential biomarkers that will be helpful in the early stage. Interestingly in this study, the author included the b two datasets: as gene expression dataset and the DNA methylation dataset. In this study, both data were initially processed, and based on the algorithm, the differentially expressed genes were identified to select the most potential one; the identified individual genes were further merged, and the overlapped genes were selected for the training of the data along with the various steps of testing and validation. The overall study suggests that this designed approach is novel, and also the protocol achieves high accuracy and will be helpful for a better tomorrow (Zhang, Li, et al., 2021).

Moreover, apart from the biomarker identification studies, researchers also used computational approaches to design the model structure, screening the potential inhibitors, vaccine design and many more (Kachhadiya et al., 2024; Mishra & Georrge, 2023; Mishra & Priya, Rai, et al., 2023; Mishra et al., 2024; Sakina et al., 2023; Vaghasia et al., 2023; Vinjoda et al., 2023).

3.3 Advances and challenges

The discovery of cancer biomarkers has become a fast-moving area with advanced technologies and methods for identifying biological markers indicative of the presence, progression, or response to treatment. With the development of high-throughput sequencing techniques like NGS, detailed analysis of genomic changes to identify new cancer-related genetic mutations and variations is possible. Single-cell sequencing techniques provide insights into tumor heterogeneity and altered cell populations, thus contributing to further elucidation of the biology of tumors. Analytical techniques like MS are shown to have applicability in almost all areas of clinical studies. It is used in the biomarker discovery in cancer diagnosis. Its application in biomarker identification and discovery of cancer has a significant impact as it analyzes protein expression profiles. Proteins are the executors of biological functions, and most biomarkers for cancer diagnosis rely on the protein biomarkers; MS technique in detecting cancer possesses a supreme role. MS combined with high-performance multidimensional liquid chromatography has identified biomarkers at the pathological process's site, followed by their detection in peripheral blood. The introduction of machine learning and artificial intelligence

(AI), provides a concrete approach in cancer biomarker identification and detection. With the application of various algorithms by AI, they can even provide real-time detection and diagnosis of brain tumors. Currently, a multi-omics approach where combined genomics, transcriptomics, proteomics, and metabolomics data is used for better understanding and prediction of cancer. The advancement of imaging technologies like PET, allows investigators to study the pharmacokinetics of anticancer drugs, identify various therapeutic targets and monitor the inhibition of these targets during therapy. Multiplex imaging techniques in cancer biomarker detection provide simultaneous detection of cancer-specific biomarkers. It includes detecting altered cancer biomarker expression levels, abnormal metabolite uptake or blood perfusion. Another technique called Liquid Biopsy utilizes non-solid biological samples like blood for detecting cancer biomarkers. This technique possesses many advantages over the traditional detection method as it is noninvasive and does not require surgery. It detects circulating tumors or DNA or the presence of exosomes in the blood for detecting cancer. It also cut the cost and diagnosis time. Thus these techniques have revolutionized cancer biomarker discovery by providing deep insights into cancer's genetic and molecular understanding, paving the way for more precise and personalized cancer diagnostics and treatments.

Besides these technological advancements, cancer biomarker identification and detection pose various challenges. Tumor heterogeneity is caused by the drastically changing environment of tumors caused by the hypoxic condition, rapidly dividing cells leading to increased mutations, and different subpopulations of cells causing cancer. Due to this intense heterogeneity in the cancer population, cancer therapy is still a challenge. Though AI has advanced in many ways in detecting cancer, further advancement in the proper interpretation of multi-omics data is required since the type and scale of different omics vary. Currently, integrating such varied data is a challenge. Handling patient data also comes with the requirement of a lot of ethical considerations. We must ensure proper consent and ethical approvals before availing the data for research and clinical applications. In addition, advanced technologies, like NGS and MS, are costly, and this surely creates a problem for low-resource settings. One of the significant challenges is to be sure that new developments in biomarker discovery will equally benefit all populations and should not worsen health conditions.

3.4 Conclusion

In the early stage, identification of the responsible biomarker is important as I can help to understand the progression and also help in the design of promising therapeutics. Researchers are using various databases, tools, and applications to speed up the progress, which is essential as the different pathogens are merging and re-emerging. The available tools and techniques are being used, and enhanced;

however, it is also important to develop the new pipeline with the advanced algorithm to gain more accuracy.

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Not applicable

Declaration of competing interest

The authors declare no known competing interests.

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CHAPTER

Genomic biomarkers: unraveling the DNA of cancer

4

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4.1 Introduction

Biomarkers, an acronym of "biological marker," are objective medical signals that can be assessed precisely and reproducibly from outside the patient. Clinical indicators differ from symptoms, which are personal perceptions of health or illness. The literature has numerous more precise biomarker definitions, which coincide advantageously. In 1998 the NIH Biomarkers Terminology Committee classified biomarkers as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Hirsch & Watkins, 2020; Parvez & Akanda, 2019). The International Programme on Chemical Safety, coordinated by the WHO, UN, and ILO, describes a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" (Biomarkers in risk assessment: validity and validation, 2001). The measurement of biomarkers varies by type. Several can be evaluated with scans, while others require a biopsy. While specimens from tumors provide unparalleled insight into tumor-immune processes, valuable biomarker information can also be obtained from more readily available areas. Recently, saliva, breath, urine, and feces have been shown to display biomarkers related to immunological and cancer cell activity. Blood is a rich source of biomarkers (Mayeux, 2004). Biomarkers have advanced cancer research in recent years. Biomarkers can measure biological processes including cancer cell presence and therapy response. These types of markers are essential to cancer research and customized treatment. Before, chemotherapy for cancer was frequently a one-size-fits-all strategy with little results. Nevertheless, the development of biomarkers has transformed the area of oncology, allowing researchers to pinpoint specific genetic abnormalities or protein expressions associated with particular kinds of cancer. This knowledge allows clinicians to personalize treatment programs for each unique patient, maximizing efficacy while avoiding adverse reactions (Oldenhuis et al., 2008). The application of biomarkers

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in clinical trials has changed cancer treatment. To examine precise genetic or molecular changes unique to each patient's tumor, physicians can determine the most effective therapy alternatives. This specific strategy not only improves patient outcomes but also decreases the danger of needless therapies and their accompanying toxicities. Biomarkers are useful in monitoring response to therapy and progression of disease. By periodically assessing the biomarker levels during medication, physicians can assess if the therapy proves helpful or if changes are necessary. Additionally, improved patient outcomes are possible as a result of real-time adjustments to treatment procedures (Uzzaman et al., 2018; Wang et al., 2011). This chapter discusses the genetic biomarkers and their immense impact on cancer research and treatment.

4.2 Genomic profiling in cancer

4.2.1 Understanding genomic biomarkers

Specific genetic abnormalities or features inside cancer cell DNA that give useful information about the disease are referred to as genomic biomarkers. They can include copy number variations, mutations, epigenetic alterations, and gene expression patterns. These biomarkers are essential to comprehending cancer's genetic landscape and creating customized cancer treatment (Nalejska et al., 2014). Genomic biomarkers are unique to each individual's cancer and can help identify the following aspects of the disease.

4.2.1.1 Diagnosis

Biomarkers that meet the requirement of proof can help restrict diagnosis. This may give rise to more specific diagnoses for particular patients. Genomic biomarkers aid in identifying the specific type of cancer and its subtypes. This is essential for tailoring treatment approaches to the characteristics of the cancer. A biomarker is a detectable material injected into an organism to test organ function or other elements of wellness. Radioactive rubidium chloride is used to assess cardiac muscle perfusion (Sarhadi & Armengol, 2022). A biomarker is a protein expression or state change that correlates with illness risk, progression, or treatment susceptibility. Prostate-specific antigen is one of the most regularly utilized biomarkers in medicine. This sign can indicate prostate enlargement, and rapid changes may indicate malignancy. Since mutant proteins can only come from tumors, selected reaction monitoring is the most extreme way to detect mutant proteins as biomarkers, offering the best specificity for medical applications (Wang et al., 2011). GTPase from KRAS oncogene is involved in numerous pathways for signaling. Precision oncology biomarkers are used to molecularly diagnose chronic myeloid leukemia, colon cancer, breast cancer, and melanoma (Nalejska et al., 2014).

4.2.1.2 Prognosis

Certain biomarkers can predict the likely course of the disease, including how aggressive it is and the chances of recurrence. Prognostic biomarkers predict patient outcomes regardless of treatment. In metastatic breast cancer research, mutant PIK3CA is a predictive biomarker. Regardless of treatment, patients with the mutation have the same prognosis. Women having mutations in PIK3CA had a poorest rate of survival before treatment. Therapeutic studies employ prognostic indicators and predictive variables to compare therapy efficacy for specific illnesses or cancers. Prognostic biomarkers, unlike predictive biomarkers, do not use explanatory variables, allowing independent disease or condition assessment (Ballman, 2015).

4.2.1.3 Treatment selection

Clinicians can select the most effective treatment by examining a tumor's genetic makeup. Targeted medicines that target cancer cells' molecular vulnerabilities have resulted. Disease-specific biomarkers are essential. A good biomarker would directly represent the illness-causing agent, be easy to detect in diverse contexts, and indicate disease activity. Biomarkers including cells, nucleic acids, and proteins are direct indications of disease agents. For instance, disease-specific nucleic acids can identify the disease's pathogen or reflect a latent infection, in which the symptoms may be caused by a different pathogen. Live cells in a sample can indicate infection, although most molecular indicators can last up to a week. However, certain indicators indirectly indicate disease-causing agents such as immunoglobulins, which are immunological responses (Van der Meide & Schellekens, 1996). In a complete blood count, basophil or eosinophil leukocytes are raised in general bacterial or viral infections, but they do not reveal the disease-causing agent (Luna Coronell et al., 2012).

4.2.1.4 Treatment monitoring

Genomic biomarkers can also be utilized to evaluate a patient's response to treatment and adapt the therapy as needed, leading to more personalized and effective care. Monoclonal protein (M protein) levels in the blood can be used as a biomarker to predict if MGUS patients are advancing to other illnesses, such as blood cancer, that may require therapy (Kyle et al., 2002). Cancer antigen 125 (CA 125) can be used to monitor ovarian cancer patients' disease status and burden during and after treatment (Gundogdu et al., 2011; Rustin et al., 2001).

4.2.2 The role of genomic profiling

Genomic profiling, the process of identifying and analyzing genomic biomarkers in cancer, has transformed the way we approach cancer diagnosis and treatment. It permits precision medicine by characterizing a patient's cancer genetic changes. Following are the key functions of genomic profiling.

4.2.2.1 Personalized treatment

Biomarkers are essential for customized therapy. Personalized medicine uses genetic and molecular profiles to manage sufferers. Clinicians can use biomarkers to identify each patient's tumor's treatment targets. This lets you select therapies with the best chance of success and the fewest negative effects. By avoiding pointless therapies, personalized medicine not only enhances patient outcomes but also maximizes the use of healthcare resources. Additionally, biomarkers are essential for predicting how a patient will respond to treatment and selecting those who will benefit most from particular medications. For instance, an EGFR mutation in non-small cell lung cancer indicates that EGFR inhibitors such as erlotinib will be well-received. Clinicians can identify patients who will benefit from focused therapy by assessing this biomarker (McVeigh et al., 2018).

4.2.2.2 Identification of therapeutic targets

By pinpointing specific genetic alterations, genomic profiling reveals potential therapeutic targets. This information has led to targeted medications that are designed to inhibit or modulate these specific molecular pathways. The leading cause of cancer fatalities globally is lung cancer. Despite extensive research on lung cancer treatment, the 5-year survival rate remains below 20%. Cancer genome heterogeneity is a key obstacle to improving survival. Lung cancer treatment with targeted therapy, especially EGFR-targeting medicines, has shown promise. This study identified medication response biomarkers by analyzing lung cancer cell line gene expression profiles. The strongest EGFR inhibitor is ZD-6474. Gene expression profiles can serve as possible predictive biomarkers for forecasting patients' reactions to medications, and treatment strategies for various individuals may be enhanced by considering their inherited histories (Wang et al., 2015).

4.2.3 Historical perspective

The history of genomic profiling in cancer is a story of scientific and technological advancement. It has evolved significantly over the years, driven by breakthroughs in genetics and molecular biology. The timeline of genomic profiling in cancer includes key milestones such as the discovery of the first oncogenes, the advent of DNA sequencing technologies, and the Human Genome Project generated a detailed map of the human genome. Historically, cancer diagnosis and treatment were primarily based on histological observations and clinical parameters. Understanding that genetic changes serve a critical role in cancer formation and progression has completely transformed the science. The phrase "biological marker" was established in the 1950s (Porter, 1957). In 1987 the definition of biological markers was given as "indicators signaling events in biological systems or samples" in three categories: marker exposure, impact, and susceptibility. Shugart and McCarthy (1990) defined biomarkers as "measurements at the molecular, biochemical, or cellular level in either wild populations from

contaminated habitats or in organisms experimentally exposed to pollutants that indicate toxic chemical exposure and the magnitude of the living thing's reaction". Van Gestel and Van Brummelen (1996) tried to develop biomarkers to distinguish them from bioindicators. According to their theory, biomarkers should only indicate sublethal metabolic changes caused by xenobiotic exposure.

4.3 Types of genomic biomarkers

Genomic biomarkers are crucial to cancer research and treatment, revealing the genetics of this complicated disease. In this chapter, we delve into various types of genomic biomarkers, beginning with the fundamental distinction between germline and somatic mutations.

4.3.1 Germline mutations

Mutations in the germline are genetic abnormalities in the DNA of an individual's germline cells, such as eggs or sperm, and are inherited from one generation to the next. Mutations can raise the risk of cancer. BRCA1 and BRCA2 germline mutations enhance both ovarian and breast cancer risk. To assess familial cancer risk and apply preventative interventions like extra surveillance or risk-reducing surgery, germline mutations must be identified (Gong et al., 2021).

4.3.2 Somatic mutations

Somatic mutations that occur in nongermline cells are caused by factors such as environment, aging, or replication of DNA mistakes. Cancer genesis and progression are driven by somatic mutations, which cause unregulated proliferation of cells and malignancies. Certain somatic mutations in a tumor can inform therapy decisions since targeted medicines can be customized to cancer cell biology. Chemicals, electromagnetic radiation, and intracellular free radical generation can damage cells and cause changes in DNA (Luzzatto, 2011; Vijg, 2014).

4.3.3 Single nucleotide polymorphisms

Chemicals, electromagnetic radiation, and intracellular free radical generation can damage cells and cause changes in DNA. Population-wide single nucleotide polymorphisms (SNPs) exist in DNA sequences. Individual SNPs may not cause cancer, but they can increase vulnerability. On the other hand, some SNPs increase cancer risk, while others prevent it. Large-scale genome-wide association studies can identify these SNPs and assess their potential for cancer susceptibility. Some

SNPs enhance the risk of skin cancer from UV light, whereas others affect lung cancer from nicotine use. SNPs in vulnerability to cancer must be understood for customized medication and risk evaluation (Bell, 2002).

4.3.4 Copy number variations

Copy number variations (CNVs) are genomic biomarkers that change the quantity of copies of certain DNA regions in the genome of a person. CNVs can be DNA sequence expansions or reductions. Variations can greatly affect cancer growth and severity. CNV-induced gene amplifications can overexpress cancer-promoting enzymes. In breast cancer, HER2 gene amplification is a well-known CNV that promotes tumor development. However, CNVs can cause tumor suppressor gene deletions, enabling unregulated division of cells. CNVs in genomes associated with cancer can reveal therapeutic goals and predict results for patients (Pös et al., 2021).

4.3.5 Structural variations

DNA segment arrangement changes cause genome structural differences. These genetic biomarkers include translocations, inversions, and significant reductions or repetitions. Due to structural differences, two genes can merge improperly, producing hybrid proteins with carcinogenic characteristics (van Belzen et al., 2021). Cancer is structurally diverse. The hereditary burden of tumors increases during tumor genesis, development, and treatment obstruction. Structural variations can disrupt, enhance, and merge cancer-related genes or reprogram noncoding DNA regulatory areas to alter the expression of genes (Cosenza et al., 2022).

4.4 Genomic biomarkers in cancer diagnosis

Genomic biomarkers have transformed cancer diagnosis by enabling earlier and more precise identification. This chapter discusses genetic biomarkers' importance in the identification of cancer, focusing on preliminary identification and assessment, genetic testing, and fluid sampling (Braakhuis et al., 2004).

4.4.1 Early detection and screening

Successful cancer therapy typically depends on early identification. Genomic biomarkers allow for precise and sensitive screening techniques that can detect cancer promptly. These methods comprise genetic examinations and scanning to detect cancer before indications appear. Breast cancer screening is a popular use of early detection genetic biomarkers. The detection of genetic abnormalities like BRCA1 and BRCA2 allows higher-risk patients to be tracked more closely, perhaps

resulting in earlier therapies and increased recovery percentages (Braakhuis et al., 2004). Removal of BRCA1/2 may result in HR deficiency in breast, ovarian, and other tumors. Currently discovered and clinically implemented poly (ADP ribose) polymerase inhibitors demonstrate the possibility to use DNA repair–directed tailored medicines to synthetically kill HR-deficient malignancies. Small molecules that block many other DNA repair proteins, such as kinases involved in the DNA destruction response (like ATM and ATR), are being tested in tumors that do not repair DNA well and tumors that do repair DNA well. These molecules are being tested on their own and with traditional agents that damage DNA (Stover et al., 2016). In a comparable manner colorectal and head-and-neck squamous cell carcinoma genetic markers, such as APC and TP53 simultaneously, enable individuals with a familial risk to undergo regular colonoscopies or other screening procedures. Insufficient APC tumor suppressor action leads to β-catenin buildup, nuclear translocation, and proliferative expression programmers. Colorectal adenomas and carcinogenesis often begin with APC mutations. Mutational activation of KRAS cannot cause cancer in vivo; however, its combination with APC mutant KRAS promotes tumor growth (Kudryavtseva et al., 2016). TP53 was commonly mutated in head and neck squamous cell carcinoma (HNSCC) before genome sequencing, with contemporary series estimating that 73% of cases have a mutation. TP53, a tumor suppressor gene, controls hundreds of downstream target genes in response to cellular stressors as a transcription factor. The 393 amino acids and 4 domains include a highly conserved DNA binding domain. It is crucial to cellular processes like DNA damage response and oncogenic stress. DNA damage activates p53, which arrests the cell cycle and attempts to repair the damage, which can lead to apoptosis or senescence. TP53 mutations are found in pre-malignant lesions and early in HNSCC pathogenesis, suggesting some participation during initial cancer development. Early detection of cancer through genomic biomarkers not only increases the likelihood of successful treatment but also reduces the overall healthcare burden associated with advanced-stage cancers (Boyle et al., 1993). Nearly 20% of HNSCC had mutations or copy number changes in oxidative stress genes, and 90% have NRF2 overexpression by IHC. The NFE2L2 locus encodes NRF2, a transcription factor that activates the cellular antioxidant reaction. It is normally expressed in the cytoplasm and destroyed by KEAP1 and CUL3. CUL3 is part of an E3 ubiquitin ligase complex that uses KEAP1, a substrate-specific adapter, to ubiquitinate and degrade NRF2. The Neh2 and Kelch/DGR domains of NRF2 and KEAP1 mediate their interaction. Oxidative stress inhibits KEAP1-NRF2 interaction, causing NRF2 to accumulate in the cytoplasm, translocate to the nucleus, and activate its target transcriptionally. NRF2 activation may enhance cancer cell proliferation and protect against cytotoxic treatment (Akanda & Hasan, 2021; Jaramillo & Zhang, 2013).

4.4.2 Molecular diagnostics

Molecular screening techniques employ genomic biomarkers to identify tumor intrinsic features. These indicators can reveal tumor kind, prediction, and medication possibilities. Some of the key genomic biomarkers in molecular diagnostics include gene mutations, gene expression profiles, and epigenetic modifications (Cerami et al., 2012). For instance, EGFR mutations in lung, head, and neck squamous cell cancer can determine the applicability of specific treatment. Typical eliminating alterations are sequence shifts, nonsense, and splice site changes. Up to 80% of tumors may have this gene inactivated due to epigenetic changes. The HER/erbB family receptor tyrosine-kinase EGFR is a key cancer target. Upon engaging its ligand, EGFR homodimerizes or heterodimerizes with other ErbB family members, activating its tyrosine kinase and autophosphorylating cytoplasmic tail tyrosine residues. This can activate downstream signaling cascades including RAS/RAF/MAPK, PI3K/AKT/mTOR. Regulation of growth by these mechanisms, invasion, blood vessel development, and metastasis (Hynes & Lane, 2005). Gene expression profiling, as seen in the Oncotype DX test for breast cancer. Oncotype DX Breast Recurrence Score evaluates genes that affect early-stage breast cancer chemotherapy and activity. It helps clinicians predict early-stage estrogen receptor-positive breast cancer recurrence. The Oncotype DX Breast Recurrence Score Test and other cancer features can aid determine chemotherapy for early-stage, hormone receptor-positive, HER2-negative breast cancer (McVeigh et al., 2014). Epigenetic changes, such as DNA methylation patterns, can reveal important insights into the behavior of cancer cells. High-resolution (450 K) DNA methylation study utilized whole blood from 15 breast cancer-discordant twin pairs. It detected 403 CpG sites with variable methylation, including potentially new breast cancer genes. A separate confirmation cohort of 21 twin pairs, DOK7 was confirmed as a blood-based cancer diagnostic candidate. Primary breast cancer tissues and cell lines had promoter DNA hypermethylation. Hypermethylation of DOK7 years before tumor detection suggests a function as a potent epigenetic blood-based biomarker and sheds light on breast cancer pathophysiology. Molecular diagnostics allow for a more precise and personalized approach to cancer treatment, minimizing unnecessary treatments and maximizing the chances of therapeutic success (Heyn et al., 2013).

4.4.3 Liquid biopsies

Liquid biopsies represent a groundbreaking development in cancer diagnosis. These tests utilize genomic biomarkers in the form of circulating tumor DNA (ctDNA), RNA, or proteins found in a patient's blood. Liquid biopsies provide a non-invasive means of monitoring cancer, tracking its progression, and identifying genomic alterations associated with resistance to treatment (Gerlinger et al., 2012). For example, liquid biopsies can detect the presence of ctDNA fragments shed by tumors in the bloodstream. These fragments carry genetic information that mirrors

the mutations present in the tumor. Monitoring changes in ctDNA levels and genetic alterations over time can offer real-time insights into a patient's reaction to therapy and drug-resistant mutations. CtDNA screening of genetic alterations is sensitive and specific, suggesting that it may improve tumor diagnosis systems, even enabling early identification. Furthermore, ctDNA analysis can correctly predict tumor development, prognosis, and targeted therapy. Using ctDNA as a liquid biopsy may revolutionize tumor care (Cheng et al., 2016). Metastatic Non-small cell lung cancer treatment is based on two parameters: tumor tissue molecular or genetic abnormalities. Tissue analysis has limits. Tissue biopsy might put the patient at danger since the tumor's position may prevent appropriate tissue collection, and researching tumor heterogeneity takes several biopsies, which is ethically and practically impossible (Rubis et al., 2019). Imaging for treatment response is problematic, especially with immunotherapy. A radiologically enlarged tumor mass indicates pseudo-progression. It is caused by leukocyte infiltration and improves long-term survival. Anti-PD-1/PD-L1 treatment can cause pseudo-progression in 6% of metastatic NSCLC. The Response Evaluation Criteria in solid tumor scoring were changed for radiographic immunotherapy patient monitoring in 2009 to solve this issue (Herbreteau et al., 2019). Since liquid biopsies allow repetitive sample, they may help identify and monitor NSCLC patients. It can identify cancer biomarkers in blood, urine, saliva, feces, and breath. A liquid biopsy sample comprises cfDNA, CTCs, EVs, cancer, and healthy tissue epigenetic signatures (van Delft et al., 2020). Biomarkers bTMB and cfDNA hold promise for cancer detection and therapeutic assessment, while PD-L1 testing and exosomal PD-L1 status signal responses to treatment (Duruisseaux et al., 2018).

4.5 Genomic biomarkers in cancer prognosis

Genomic biomarkers are essential for cancer prediction, advancement of the disease, forecasting, and therapy results (Ali Syeda et al., 2020).

4.5.1 Predicting disease progression

Genomic biomarkers are used in cancer's outlook to predict the course of the disease. Clinicians can use genetic changes in cancer cells to predict the extent of tumor growth and spread. Genetic mutations, gene expression, and epigenetic alterations are biomarkers. Nevertheless, predictive indications are increasingly used to evaluate the clinical advantage, advancing customized treatment (van Gool et al., 2017). Mutations in the TP53 gene in numerous cancer types might enhance genomic instability as well as therapy obstruction, resulting in a worse prognosis. In contrast, genetic indicators like low Ki-67 expression in breast cancer may indicate a less malignant tumor and a better outlook (Fattore et al., 2019). The miRNAs upregulated in squamous cell carcinomas of the head and neck carcinoma,

particularly vocal malignancies, indicate start, progression, and prediction, are connected with radiochemotherapy and resistance to heat, and are linked to HPV and lifestyle (Sohel, 2020). Triple-negative breast cancer is diverse, aggressive, has a high distant recurrence risk and poor prognosis. BRCA1/2 and protein biomarkers are utilized to prognosticate TNBC and stratify patients for responsiveness to the expanding number of new targeted or immunotherapy treatments. NAA10, emerging protein N-terminal amino groups, and inner protein residues of lysine have been investigated by Kim et al. (2019). The scientists say NAA10 controls the growth of cells, distinction, movement, autophagy, and death. Also, NAA10 amplification in several cancer types was linked to lifespan and recurrent (Kim et al., 2019). Still, melanoma diagnostic and prognosis indications depend on miRNA instability. MiRNAs control MAPK signaling or immunological checkpoint expression to predict melanoma cell response to targeted and immunological treatments, which makes melanoma cells resistant to MAPK and immune inhibitors of checkpoints (Huber et al., 2018).

4.5.2 Assessing treatment outcomes

Genomic biomarkers are crucial to cancer therapy results. During treatment, doctors can assess response to therapy as well as recognize mechanisms of resistance by examining tumor DNA, RNA, or protein levels. New tumor genome mutations can indicate resistance to particular therapy. These gained genetic modifications may reduce the efficacy of the original therapy, requiring modifications. Monitoring genomic biomarkers throughout the treatment journey can help detect these changes early, enabling timely intervention and improved treatment outcomes (Ciardiello & Tortora, 2008). EGFR-targeted therapies are used to treat several cancers, including HNSCC. In locally advanced and metastatic conditions, cetuximab, a chimeric murine/human monoclonal antibody against EGFR, improves overall survival relative to standard treatment, but its use is disputed. However, two humanized EGFR antibodies have not improved overall survival in recurrent/metastatic or locally advanced settings. Also disappointing are gefitinib and erlotinib, two first-generation EGFR tyrosine kinase inhibitors (Vermorken et al., 2013).

4.5.3 Clinical implications

The clinical implications of genomic biomarkers in cancer prognosis are profound, revolutionizing the landscape of oncology. They aid clinical choices, guidance for patients, and personalized cancer care. Genomic biomarkers aid clinicians in normal practice:

- Choose the best treatment for each patient based on their genomic profiles.
- Detect and control therapeutic adverse effects using specific to patient's genomics.

- Provide personalized prognostic data to help patients make choices regarding healthcare.
- Better surveillance of patients throughout and following treatment allows early illness reappearance or development diagnosis.
- Target treatments to those most likely to benefit from utilizing healthcare resources (Sausen et al., 2015).

Hence Genomic biomarkers in cancer prognosis help forecast the course of the disease, evaluate therapy outcomes, and influence clinical choices. Integrating them into oncology improves accuracy and performance, boosting cancer patients' standard of life and survival rates. These biomarkers provide cancer patients and doctors hope and individualized treatments (Berger & Mardis, 2018).

4.6 Genomic biomarkers in cancer treatment

The field of precision medicine utilizing genomic biomarkers improves cancer therapy and personalization.

4.6.1 Targeted therapies

The discovery of genetic biomarkers in cancerous cells enables tailored medicines, a revolutionary method for treating cancer. These medicines disrupt chemicals or processes that fuel cancerous cell development and viability while protecting cells from damage. Genomic biomarkers including mutations in genes or amplification may determine patients who are going to benefit from targeted medicines. Regarding lung cancer, EGFR mutations are genetic biomarkers for erlotinib effectiveness. In breast cancer, the overexpression of HER2 is a biomarker for drugs like trastuzumab. Targeted therapies offer the potential for improved treatment responses, fewer side effects, and better patient outcomes (Shen, 2011). Functional biomarkers include altered DNA repair. Cancer cells, like normal cells, experience endogenous and external DNA damage. Multiple DNA repair processes must function properly for cancer cells to proliferate and resist therapy. Genomic instability and cancer result from defective DNA repair, yet targeted therapy is possible (Shen, 2011). Filamin-A (FLNA), also called as actin binding protein 280 (ABP-280), forms orthogonal actin networks and fibers for stress. Filamin-A scaffolds cytoplasmic and nuclear signaling proteins and docks transmembrane proteins to actin. Relevant, filamin-A interacts with non-cytoskeletal proteins of distinct functions and is involved in independent processes, according to several studies. Human genetic disorders and cancers have filamin-A mutations and abnormal expression including metastasis and DNA damage (Yue et al., 2013).

4.6.2 Immunotherapies

Immunotherapies harness the power of the immune system to combat cancer. Genomic indicators can identify immunotherapy-responsive patients. Many biomarkers have been used in clinical practice, but others are still being studied. Cancer cells and other tumor cells that express PD-L1 can block "killer" T lymphocytes from destroying tumors via the PD-1 receptor. The CRI-SU2C Dream Team was one of the first to link PD-L1 expression to PD-1/PD-L1 checkpoint immunotherapy responses in patients with different cancers. Thus, tumor PD-L1 levels have been utilized as biomarkers to predict which patients may benefit from immunotherapies. Patients with high PD-L1 expression are more likely to respond to immunotherapies, while those without PD-L1 can still respond due to its dynamic nature. The tumor may "turn on" PD-L1 expression later to shield itself from killer T cells, even if it is not expressing it now (Janis et al., 2014). Malignant tumors have genetic abnormalities that cause most of their life-threatening symptoms, such as constant growth and migration. Mutated proteins can make tumors "stand out" and give neoantigens for the immune system to assault cancer cells. The number of mutations accumulated by a tumor is called tumor mutational burden (TMB) biomarker. A tumor with more mutations is more likely to express altered proteins and neoantigens that the immune system can target. As a result, patients with malignancies with elevated TMB have been discovered to be substantially more likely to react to circuit therapy. Preexisting immunological responses and PD-1/PD-L1 expression are linked to high TMB. This study found that tumor genetics affect anti-CTLA-4 checkpoint therapy outcomes (Tumeh et al., 2014). Certain tumors decrease their DNA repair ability, resulting in severely mutated tumors with high microsatellite stability (MSI-hi) and inadequate repair of mismatches. MSI-hi/dMMR cancers are typically invaded by killer T cells and express PD-L1, making them ideal for PD-1/PD-L1 checkpoint immunotherapy. Anti-PD-1 immunotherapy became the only approved treatment for MSI-hi/dMMR solid tumors in May 2017. Individual mutations are promising cancer immunotherapy biomarkers. Unfortunately, two CRI-funded research found tumor alterations that caused primary and acquired immunotherapy resistance (Shin et al., 2017). Tumor gene expression often dysregulates, causing them to produce normal proteins abnormally and develop neoantigens from mutations. These also attack cancer cells immunotherapeutically. HER2, a growth-related protein found on healthy cells, is typically overexpressed in cells with cancer. Multiple targeted immunotherapies for HER2+ tumors have been authorized. Another protein, NY-ESO-1, is only expressed in fetal and adult testicular cells. Cancer cells can accidentally reinstate NY-ESO-1 production. NY-ESO-1 expression has been linked to more aggressive ovarian cancer. A CRI-funded study found that a vaccination targeting NY-ESO-1 increased survival in aggressive ovarian cancer patients. Finally, HPV and EBV-infected tumors can express aberrant viral proteins that can be used by the immune system for immunotherapy (Sivan et al., 2015).

4.6.3 Personalized medicine

Personalized medicine uses genomic biomarkers to customize cancer treatment for every individual with cancer. The importance of biomarkers in customized therapy is huge. Based on genetic and molecular traits, personalized medicine tailor's treatment regimens to each patient. This goal requires biomarkers (Pepe et al., 2003). Biomarkers help clinicians find tumor-specific treatment targets. This helps choose the most effective medicines with the fewest side effects. By eliminating unneeded therapies, personalized medicine improves patient outcomes and optimizes healthcare resources. Biomarkers also help predict treatment response and identify people who will benefit from specific medications. EGFR mutations in non-small cell lung cancer suggest a good response to gefitinib. Clinicians can identify patients who will benefit from focused therapy by assessing this biomarker. The concept of personalized medicine extends beyond treatment selection. It also encompasses personalized dosing, monitoring, and treatment adjustments patient genomic response. This method may improve cancer patients' treatment outcomes and quality of life (Matsui, 2013).

4.7 Case studies

In this chapter, we explore real-world case studies that illustrate the practical applications of genomic biomarkers in understanding, diagnosing, and treating cancer. The focus is on three major cancer types: breast cancer, and lung cancer, each with its unique genomic biomarkers and implications for patient care illustrated in Fig. 4.1.

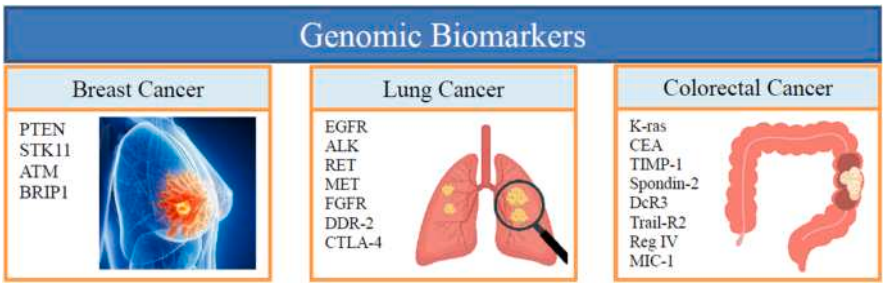

FIGURE 4.1

Genomic biomarker of breast, lung, and colorectal cancer.

4.7.1 Breast cancer genomic biomarkers

Breast cancer is one of the most studied cancers and genomic biomarkers have made major contributions to our understanding of this disease. Several well-known genomic biomarkers have shaped the management of breast cancer:

4.7.1.1 *PTEN/STK11*

Some constitutional syndromes with overt symptoms and "biomarkers" enhance breast cancer risk, such as PHTS and Peutz-Jeghers disorders. Cowden syndrome primarily affects women with breast, endometrial, follicular thyroid, numerous gastrointestinal hamartomas, macrocephaly (> 97%), and mucocutaneous lesions (Stratton, 1996).

4.7.1.2 *STK11*

Individuals with PJS are more likely to develop colon, breast, and ovarian cancer. Most mutations are modest deletions, small base alterations that alter protein function and kinase activity. Among almost 400 patients, nearly 300 had STK11 mutations the likelihood of developing breast cancer by age 60 was 50% (Hearle et al., 2006).

4.7.1.3 *ATM*

ATM is a gene that produces a DNA-repairing protein. Bi-allelic and may be mono-allelic genetic alterations produce ATM phenotypes. In Ataxia-Telangiectasia, two mutations cause a weakened immune system illness and predisposition to leukemia and lymphoma. Individuals with a single ATM gene mutation may or may not have a higher risk of breast cancer (Meyer et al., 2007).

4.7.1.4 *BRIP1*

BRIP1, also known as FANCJ, like PALB2, causes heterozygous and homozygous illness. The BRCA interacting helicase is BRIP1. Constitutional bi-allelic mutations in these genes cause Fanconi anemia. Constitutional heterozygous carriers may have a higher breast cancer danger, according to one study (Seal et al., 2006).

4.7.2 Lung cancer genomic biomarkers

Genomic indicators have revolutionized lung cancer diagnosis, a difficult illness with several subtypes:

4.7.2.1 *EGFR mutations*

The epidermal growth element receptor is an ERBB tyrosine kinase receptor. The EGFR gene is on chromosome 7 short arm at 12. The external ligand homo- or heterodimerizes EGFR, phosphorylating cytoplasmic tyrosine kinase sites and activating intracellular pathways such PI3K/AKT/mTOR and RAS/RAF/MAPK,

which induce cell proliferation, metastasis, apoptosis (Sholl, 2015). EGFR mutations are found in 10% of US lung cancer patients and 30% to 50% in East Asia. Erlotinib, gefitinib, and osimertinib raise EGFR TKI sensitivity. NSCLC patients' survival and quality of life have improved with EGFR mutation-targeted therapy (Sharma et al., 2007).

4.7.2.2 Anaplastic lymphoma kinase

Anaplastic lymphoma kinase (ALK) is an insulin receptor superfamily tyrosine kinase receptor. The short arm of chromosome 2 contains the ALK gene at location 23 (Zhao et al., 2015). ALK Gene reorganization was first found in anaplastic large cell lymphoma, later in a subset of NSCLC cancers with an ALK-EML4 gene fusion. A chimeric protein having inherent kinase activity enhances malignant development and multiplication after this rearrangement. ALK-positive patients can benefit from ALK inhibitors like crizotinib, ceritinib, or alectinib. These targeted therapies have proven effective in slowing down disease progression and extending survival (Chatziandreou et al., 2015).

4.7.2.3 RET proto-oncogene

The RET proto-oncogene is located at position 11.2 on the long arm of chromosome 10. The tyrosine kinase receptor for the glial cell line-derived neurotrophic factor family of ligands is produced by this and is responsible for cell proliferation, migration, and differentiation (Knowles et al., 2006).

4.7.2.4 MET proto-oncogene

The MET gene is located at position 31 on the long arm of chromosome 7. This oncogene encodes a tyrosine kinase receptor that activates signaling pathways essential for cell proliferation and survival, motility. Mutation, protein overexpression, and gene amplification activate MET pathologically. MET changes were originally reported in renal papillary carcinoma patients, and MET kinase domain mutations activated the receptor constitutively (Schmidt et al., 1997). In 3% of squamous cell lung malignancies, 8% of lung adenocarcinomas, extracellular semaphorin and juxtamembrane domain MET mutations are identified. MET amplifications are detected in 4% of lung adenocarcinomas and 1% of squamous cell lung cancers and are associated with MET inhibitor sensitivity (Paik et al., 2015).

4.7.2.5 Fibroblast growth factor receptor

The fibroblast growth factor receptor (FGFR) gene encodes a FGFR family tyrosine kinase receptor at location 12 on chromosome 8. The FGFR family has 4 receptor tyrosine kinases. Upon ligand-receptor interaction, FGFR dimerizes and phosphorylates FRS2α, activating pathways such as RAS/MAPK, PI3K/AKT/mTOR, boosting survival of cells, movement, invasiveness, growth (Jiang et al., 2015). In lung cancer, FGFR1 amplification is more common in current smokers

and in squamous cell carcinoma (20%) than adenocarcinoma (3%). Additional clinic-demographic factors also predict FGFR1 amplification (Dienstmann et al., 2014).

4.7.2.6 Discoidin domain receptor tyrosine kinase 2

At location 23.3 on the long arm of chromosome 1, this encodes a mesenchymal-expressed receptor, it acts as a ligand to attach fibrillar collagen. Discoidin domain receptor tyrosine kinase 2 (DDR2) enhances cell migration, proliferation, and survival by activating SRC, SHC, JAK, ERK1/2, and PI3K (Payne & Huang, 2014). DDR2 mutations are found in 3%–4% of lung squamous-cellular cancers and 0.5% of adenocarcinomas (An et al., 2012).

4.7.2.7 Cytotoxic T-lymphocyte-associated antigen 4

Inhibitory monoclonal antibodies stop cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) from binding to its ligands (CD80/CD86), which activates the anticancer defense made by certain T cells (Seetharamu et al., 2016). In a phase 2 study of advanced NSCLC patients receiving ipilimumab and chemotherapy, PFS improved significantly compared to a control group receiving treatment solely. A phase 3 trial of ipilimumab and chemotherapy in squamous histology NSCLC patients is underway (Carrizosa & Gold, 2015).

4.7.3 Colorectal cancer genomic biomarkers

Colorectal cancer is another cancer type where genomic biomarkers are pivotal in guiding patient care:

4.7.3.1 K-ras

The Ras family protein K-ras binds guanine nucleotides and participates in signal transduction inositol-3-kinase and serine/threonine protein kinase B pathways. 40%–50% of spontaneous colon tumors and adenomas have K-ras alterations (Fearon & Vogelstein, 1990).

4.7.3.2 CEA

CEA is a molecularly heavy compound immunoglobulin superfamily glycoprotein. A hydrophobic region on CEA's carboxy-terminal is changed to bind glycosyl phosphatidylinositol to the surface of the cell. It is present in histology specimens, but generally in serum. This protein has long been utilized as a biomarker for CRC and other malignancies (Søreide et al., 2009).

4.7.3.3 Tissue inhibitor of metalloproteinase type 1

Multipurpose glycoprotein TIMP-1 suppresses many extracellular metallic proteins. CRC patients had far higher plasma TIMP-1 levels than safe donors of blood, who

have a restricted fluctuation. Importantly, it can detect early CRC (Holten-Andersen et al., 1999).

4.7.3.4 Five-serum-marker panel (Spondin-2, DcR3, Trail-R2, Reg IV, MIC 1)

Recent studies examined 600 serum samples for four serum biomarkers: DcR3, spondin-2, Reg IV, TRAIL-R2. CRC patients had higher levels of all four markers and MIC 1, in comparison to individuals with benign diseases and regular controls (Roessler et al., 2006). Additional Relevant Biomarkers are substances: Habermann found that C3a-desArg is considerably greater in serum from colorectal adenomas in cancer patients than in healthy persons. Only C3a-desArg was able to predict CRC with 97% sensitivity and 96% specificity in a blinded validation analysis (n=59) (Habermann et al., 2006). Glycolytic pyruvate kinase M2. Some unknown process releases this cytosolic enzyme into circulation. Dying cancer cells may secrete M2-pyruvate kinase. Two distinct studies revealed that M2-pyruvate kinase detects CRC with 48%–58% sensitivity and 90%–95% specificity. Combining M2-pyruvate with CEA boosts its sensitivity without lowering its level of specificity (Schneider et al., 2005).

4.8 Challenges and limitations of using biomarkers in cancer research

In cancer research, biomarkers encounter obstacles and limitations despite their importance. Finding reliable and clinically meaningful biomarkers is difficult. Biomarkers must be discovered and validated through intensive study and testing to ensure accuracy and predictive usefulness. Biomarker analysis is also complicated and expensive, restricting its use in certain areas of healthcare. Another drawback is tumor heterogeneity. Tumor cell groups have distinct genetic and molecular traits. Therefore, investigators are constantly studying biomarkers or composite biomarker arrays to better determine eligibility for therapy (Taube et al., 2009). Biomarkers can change response to therapy or progression of the illness. Therapy adjustments require biomarker analysis. This may be technically challenging and require frequent testing. Several genetic indicator tests are expensive and not available for many patients, especially in resource-limited healthcare environments. Biomarkers in cancer care may not reach more people due to the high cost of sequencing and other advanced technologies for diagnosis. It can be difficult to coordinate and reproduce biomarker analysis across labs and organizations. Different methods of testing and their interpretation can affect outcomes dependability and uniformity (Ileana Dumbrava et al., 2018). Research, investment in technology and infrastructure, and a dedication to making biomarker testing more accessible and affordable are needed to deal with these issues. Furthermore, legal genetic information utilization and patient privacy must

be improved. Genomic biomarkers are crucial to cancer research and treatment, presenting the opportunity for improved, customized, and focused care despite these limitations (McDermott et al., 2013).

4.9 Future directions in genomic biomarkers

Biomarkers are substances in the treatment of cancer that will benefit from study and innovation. Clinical biomarker research improves the results for patients and optimizes therapy. Developing biomarkers with predictive power for determining people most likely to respond to drugs is one approach. By predicting responses to therapy and preventing unsuccessful therapies and negative outcomes, clinicians conserve money as well as time. This customized approach ensures patients receive the finest treatment from the start (Fenech, 2002). Multiple biomarkers or composite panels may enhance the course of treatment and diagnosis. More indicators help oncologists comprehend the malignancy's hereditary and molecular features, allowing more customized chemotherapy. Genomic studies may help detect cancer-prone people. By leveraging genomic information, healthcare providers can develop targeted surveillance and prevention strategies, offering individuals the opportunity for early interventions that can reduce cancer risk. Future directions in genomic biomarkers will also involve a more holistic approach to cancer care. Beyond disease-specific biomarkers, there will be a focus on patient-reported outcomes and quality of life. Understanding the impact of genomic-driven treatments on patients' well-being, both physically and emotionally, will be critical in shaping future cancer care strategies. Additionally, biomarker research alliances and activities are essential for progress. Researchers can speed biomarker discovery and validation by sharing resources and knowledge, improving patient care. Translating biomarker findings into clinical practice requires collaboration between academics, industry, and regulators (Vincent et al., 2020).

4.10 Conclusion

Biomarkers transformed cancer research and enabled individualized medicine. These metrics reveal tumor features, therapy response, and patient outcomes. Biomarkers allow clinicians to customize treatment programs for each patient, improving efficacy and reducing negative effects. Biomarker research evolves with technology and collaboration. Biomarkers in cancer treatment could enhance patient outcomes and optimize treatment options. Researchers, physicians, and industry partners can speed biomarker discovery and clinical use through partnerships and projects. To fully use biomarkers to fight cancer, we must invest in research, technology, and cooperation.

Abbreviation

WHO	World Health Organization
DNA	Deoxyribonucleic Acid
KRAS	Kirsten rat sarcoma viral oncogene homolog
GTPase	Guanosine triphosphate hydrolases
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
MGUS	Monoclonal gammopathy of undetermined significance
CA 125	Cancer antigen 125
EGFR	Estimated glomerular filtration rate
BRCA1	Breast Cancer gene 1
SNP	Single nucleotide polymorphism
CNVs	Copy number variations
HER2	Human epidermal growth factor receptor 2
ADP	Adenosine diphosphate
ATM	Ataxia telangiectasia mutated gene
ATR	Ataxia telangiectasia and Rad3
HNSCC	Head and neck squamous cell carcinoma
APC	Antigen presenting cell
TP53	Tumor protein 53
IHC	Immunohistochemistry
NRF2	Nuclear factor erythroid 2-related factor 2
KEAP1	Kelch-like ECH-associated protein 1
CUL3	Cullin 3
erbB	Erythroblastic leukemia viral oncogene homolog
RAS	Rat sarcoma virus
RAF	Rapidly Accelerated Fibrosarcoma
MAPK	Mitogen-activated protein kinase
PI3K	Phosphoinositide 3-kinases
AKT	Ak strain transforming
mTOR	Mammalian target of rapamycin
JAK	Janus kinase
STAT	Signal transducer and activator of transcription
CpG	5' C phosphate G 3'
DOK7	Docking Protein 7
ctDNA	circulating tumor DNA
NSCLC	Non-small cell lung cancer
PD-L1	Programmed Cell Death Ligand 1
RECIST	Response Evaluation Criteria in Solid Tumors
iRECIST	Radiographic monitoring of immunotherapy receiving patients
cfDNA	Circulating cell-free DNA
CTCs	Circulating tumor cells
Evs	Extracellular vesicles
bTMB	Blood-based tumor mutation burden
Ki-67	Kiel 67
miRNA	MicroRNA
HPV	Human papillomavirus
TNBC	Triple negative breast cancer

NAA10	N-acetyltransferase 10
ALK	Anaplastic lymphoma kinase
FLNA	Filamin-A
TMB	Tumor mutational burden
dMMR	deficient mismatch repair
MSI-hi	Microsatellite stability
NY-ESO-1	New York esophageal squamous cell carcinoma 1
EBV	Epstein-Barr virus
PHTS	PTEN hamartoma tumor syndrome
PALB2	Partner and Localizer of BRCA2
EML4	Echinoderm microtubule-associated protein-like 4
RET	Rearranged during transfection
MET	Mesenchymal Epithelial Transition
FGFR	Fibroblast growth factor receptor
DDR2	Discoidin domain receptor tyrosine kinase 2
CTLA-4	Cytotoxic T-lymphocyte-associated antigen 4
CEA	Carcinoembryonic antigen
TIMP	Tissue Inhibitor of Metalloproteinase Type 1
CRC	Colorectal cancer
DcR3	Decoy receptor 3
Trail-R2	TRAIL receptor 2
MIC 1	Macrophage inhibitory cytokine 1

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CHAPTER

Proteomics and protein biomarkers in oncology

5

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5.1 Introduction

Cancer is a significant contributor to global mortality, causing 21% of all deaths. It is the second most prevalent disease in advanced nations (Ferlay et al., 2015). In 2012 there were 14.1 million new instances of cancer reported. It is projected that by 2030, the total number of new cases of cancer will increase to 23.6 million (Ferlay et al., 2015). Although there have been significant advancements in cancer research and the development of drugs, the overall prognosis for the long-term survival of the majority of cancer patients is still unfavorable (Ferlay et al., 2015). Typically, this is due to the fact that the cancers are diagnosed at a late stage and there is insufficient guidance on the optimal treatment. In order to tackle this clinical difficulty, research endeavors have been focused on identifying biomarkers that can be used for early diagnosis, prognosis, and prediction of response to treatment. Additionally, efforts have been made to develop new treatment approaches that specifically target the molecular abnormalities of the tumor. So far, genomics methods have provided significant insights into the functional capabilities of cells and have laid the groundwork for proteomics research. Proteomics enhances genomic methods by offering supplementary details on the proteins, which play a crucial role in cell activity. This includes information about their functionality, posttranslational modifications (PTMs), interactions with other biological molecules, and their reactions to environmental variables (Ocak et al., 2009). In recent years, mass spectroscopy (MS)-based proteomic technologies have allowed us to collect extensive profiling datasets with exceptional precision and resolution (Aebersold & Mann, 2016). As a result, we have entered an era where MS is being more widely used, serving as a viable alternative to conventional immune-based methods that focus on individual tumor antigens. These traditional

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approaches often suffer from low specificity and interference from matrix compounds (e.g., urine compounds that interfere with immunological detection) (Huang & Zhu, 2017). This chapter aims to evaluate the current MS-based proteomic methods used in cancer biomarker research. The research involving cancer proteomics have yielded the most reliable results, which have been applied to several aspects of cancer management. These include early cancer detection, diagnosis, patient stratification, prognosis, prediction of drug response, and treatment monitoring. Afterward, the primary findings are cross-referenced to examine their connections with the development and advancement of cancer. In conclusion, we will explore the existing limitations and challenges in the field of onco-proteomics and provide recommendations for future progress.

Biomarkers are among the crucial instruments required for diagnosis, therapy, prognosis, monitoring, and detection. According to Srinivas et al. (2001), biomarkers are biological substances that serve as both indicators of physiologic condition and of changes that occur during a disease process. A biomarker's usefulness is derived from its capacity to offer an early indication of the illness, monitor the course of the disease, facilitate diagnosis, and produce a factor that is quantifiable across populations. The first draft of the human genome (Venter et al., 2001) has accelerated the search for biomarkers and served as a catalyst for the next generation of molecular research, known as functional genomics or proteomics. The study of the entire protein complement, or the cell's proteome, is known as proteomics. The proteome is dynamic and constantly changing, in contrast to the genome, for a variety of reasons. These consist of PTMs, functional and temporal regulation of gene expression, and differential splicing of the corresponding mRNAs. Proteomic technologies make it possible to identify the protein alterations brought on by the illness process with a reasonable degree of accuracy. The fact that the discovered protein is also the biological endpoint gives proteomics an intrinsic benefit. When a healthy cell turns into a cancerous cell, specific changes take place at the protein level. These changes can impact cellular function and include altered expression, differential protein modification, changes in particular activity, and aberrant localization. Cancer proteomics' primary focus is recognizing and comprehending these alterations.

5.2 Techniques in proteomic analysis

Genomics-based methods for biomarker creation involve assessing the expression of complete sets of mRNAs, such as differential display, serial analysis of gene expression, and large-scale gene expression arrays. Nevertheless, the task of analyzing the most accurate data and customizing the findings to a specific use case continues to be difficult. While investigations into differential mRNA expression provide valuable insights, they do not consistently align with protein quantities (Srinivas et al., 2002). Proteins frequently undergo proteolytic cleavage

or posttranslational changes, such as phosphorylation or glycosylation. Strategies for discovering cancer biomarkers that focus on expressed proteins are gaining popularity due to the ability of proteomic techniques to identify the proteins, whether changed or unmodified, that play a role in cancer progression.

Two-dimensional gel electrophoresis has been the predominant method of electrophoretic technology for ten years and is the most extensively utilized technique for protein separation. Proteins on a two-dimensional gel were initially described 25 years ago. They are segregated in the first dimension according to their isoelectric points and then in a second dimension based on their molecular masses. Two-dimensional gel electrophoresis can be used to assess protein extracts from whole cells or tissues in numerous instances. Utilizing narrow, immobilized pH gradients in the first dimension enhances resolution capability and facilitates the identification of proteins with low abundance. Radioactive or fluorescent labeling and silver staining enable the visualization of several proteins in a single gel. The differences between the samples can be assessed and the relative quantities can be established by calculating the ratios of spot intensities in independent two-dimensional gels. Matrix-assisted desorption/ionization time-of-flight MS (MALDI-TOF MS) is a technique used to analyze and identify minute quantities of protein extracted from the gel (Srinivas et al., 2002). The combination of these advancements has enhanced the appeal of utilizing two-dimensional electrophoresis for analyzing intricate protein mixtures. A few of the techniques in the proteomic analysis for the biomarker of cancers are described below:

5.3 Isotope-coded affinity tags

A high-throughput method that enables direct qualitative and quantitative comparisons of complicated protein mixtures has been devised by leveraging the capability of MS. This technique uses a chemical group or label created in two different isotopic forms: heavy and light and is comparable to the microarray approach for evaluating differential gene expression between two cell states. These labels, also known as isotope-coded affinity tags, were first reported by Gygi et al. (1999). They coupled to every cysteine residue in a protein mixture. One sample (such as cancer cells) receives the addition of a heavy isotope, while the second sample (healthy cells) receives the addition of a lighter isotope. Following the combination of the samples, the proteins are broken down, and the peptides are examined in a MS. Peptide behavior during separations is unaffected by isotopic replacements. Peptides from the two distinct samples thus enter the spectrometer simultaneously. By creating and examining peptide fingerprints, the mass spectrometer is able to identify individual peptides as well as determine the relative quantity of heavy and light peptide forms in each sample. This allows for the determination of the overall protein abundance in two different states of cells or tissues. This is particularly significant for the discovery of biomarkers since it

provides us with molecular handles to target intervention techniques through expression analysis of all proteins and detection of alterations that are a function of a disease process.

5.4 LC-MS/MS

The capacity of the tandem mass spectrometer to sequence data for a particular peptide even when the sample contains several peptides is its main strength. Peptides from exceedingly complex mixtures are concentrated and separated using reversed-phase LC prior to sequencing by MS. It has been shown that LC-MS/MS improves analyte identification and separation in the sub-femtomolar range (Gygi et al., 1999). Conventional HPLC pumps and columns connected to a tandem mass spectrometer are integrated into the LC-MS/MS system. To enable effective coupling of chromatography and ion detection, the mass spectrometer and pumps are managed by the same software (Peng and Gygi, 2001). To increase sensitivity, samples can be added using a pressure cell or an injector. Precolumn traps and vented microcapillary columns are two more loading techniques (Peng and Gygi, 2001). Peptides are electro-sprayed to ionize them after being eluted from the HPLC columns, and then a tandem mass spectrometer is used to analyze the results. Based on their m/z, the peptide ions are successively expelled from an ion trap to the detector. Ions with different m/z values may be ejected from the ion trap initially after a chosen peptide of interest. In order to acquire a tandem mass spectrum with the sequence information, the retained peptide ion is broken and transmitted to the detector (Peng and Gygi, 2001). The amino acids can be identified by searching a protein or translated nucleotide database with the fragmented ions. Thus it is possible to isolate and sequence hundreds of chosen peptides from a single sample using a single LC-MS/MS scan. Finding protein targets for reactive electrophiles and mapping the adducts to particular amino acids is another benefit of this technology. This strategy can pave the way for the identification of novel biomarkers and aid in the understanding of the mechanisms by which exposure to the environment can modify proteins and start the process of carcinogenesis. In fact, congenital adrenal hyperplasia has been quickly monitored using the LC-MS/MS technology using dehydrated filter-paper specimens of blood (Lai et al., 2001).

5.5 Imaging MS

This technique examines the patterns of protein expression from target tissues by utilizing the molecular sensitivity of MS. Direct mapping of the peptides and proteins found on the surface of individual cells and tissue sections is made possible by it. Tissue slices are usually mounted on steel plates that have been treated with a

matrix solution. After drying, they are placed inside a mass spectrometer that is managed by specially designed imaging software. Next, using laser dots, a raster over the sample surface is used to create molecular pictures. The sample plate is moved for successive places, and the laser positions are fixed. For every spot, mass spectral data are obtained from the molecules on the exposed surface. Thousands of spots could be present in a typical data array, depending on the molecular weight range and required image resolution. From each location ablated by the laser, a composite of more than 200 protein peaks can be found in the mass spectrum (Stoeckli et al., 2001). From a single raster, hundreds of picture maps with different molecular weights can be produced. By highlighting the significance of particular proteins' spatial localization during carcinogenesis and neoplasia, imaging MS may improve the identification of biomarkers.

5.6 Free flow electrophoresis

Despite being a highly effective separation method, two-dimensional electrophoresis is constrained by the amount of protein that can be processed and the insolubility of some protein classes, such as hydrophobic membrane proteins. To get over the aforementioned restriction, a recently published process for resolving intricate protein combinations has been developed. This method combines sodium dodecyl sulfate–polyacrylamide gel electrophoresis, liquid-based isoelectric-focusing, and free flow electrophoresis (Hoffmann et al., 2001). Peptide fragment sequencing utilizing capillary column, reversed-phase HPLC–MS is used to identify resolved proteins. Proteins can be resolved using the incredibly potent liquid-based, isoelectric-focusing technique known as free flow electrophoresis, which has no restrictions on the volume of sample that can be put into the apparatus. It is useful in cell-mapping proteomics and has the ability to fractionate intact protein complexes. Additionally, it permits the creation of protein profiles by employing different denaturing isoelectric-focusing buffers that comprise a blend of zwitterionic and urea-thiourea detergents.

5.7 Challenges in proteomic studies

The preparation of the sample is one of the most important steps in proteomics. This is significant because the variety of proteins generated from cell populations may have an impact on repeatability. Several parameters are involved from the moment of sample collection until proteins are added for analysis. An attempt should be made to process each sample in the most comparable way feasible. To guarantee that results are directly related to the tumor under consideration, cancer cells should be liberated from stroma, necrotic tissue, contaminated serum proteins, and blood cells. Aspiration with a fine needle and surface scraping are two examples of

mechanical techniques that have been effectively employed to collect cancer cells. To separate pure populations of cancer cells, nonenzymatic techniques including immunomagnetic separation and calcium deprivation have also been employed. The invention of laser capture microdissection (LCM) has been a significant advancement in sample preparation techniques. Selected cell populations can be obtained from a portion of a complicated and heterogeneous tissue sample in a single step thanks to the LCM technique. For molecular research, LCM has been effectively utilized to isolate pure populations of cancer cells from frozen, paraffin-embedded, stained, and unstained tissues. By isolating particular cell populations, LCM enables the extraction of pure neoplastic cell populations from lesions with a diameter of less than 1 mm without allowing neighboring nonneoplastic cells to intrude. The effective isolation of matched healthy epithelial, stromal, benign, preneoplastic, neoplastic, and cancer cells from the same tissue suggests that this technique may be useful in the search for new cancer biomarkers.

Since strong data analysis tools are essential to the development of proteome technologies combining high-throughput methodologies, bioinformatic tools are essential components of proteomic analyses. The management and examination of the data required for proteomic studies encourage computational scientists, biostatisticians, and biologists to work together more frequently. Proteomic investigations require bioinformatic tools for analysis, storage, management, search, and retrieval, among other tasks. With the current proteomic platforms of arrays, MS, and two-dimensional gel electrophoresis in mind, bioinformatic tools are being created. Software programmes with intuitive user interfaces for linearization and merging of scanned pictures, segmentation and identification of protein spots on the images, matching, and editing are among the tools designed to analyze patterns from two-dimensional electrophoresis protein analysis. Progenesis (Nonlinear Dynamics), ImageMaster (Amersham Biosciences), Melanie 3 (GeneBio), PDQuest 2-D Analysis Software (Bio-Rad Laboratories), ProteinMine (Scimagix), and the Z3 2D-Gel Analysis System (Compugen Limited) are some of the commercial two-dimensional image analysis tools available to the proteomics community. Certain software programmes can work with automated robotic equipment to remove certain regions from the gel so that they can be subjected to further MS analysis. In proteomic research, the ability to interface image analysis software with database tools for picture storage is crucial, although many commercial systems fall short in this regard (Chakravarti et al., 2000).

This problem is starting to be addressed by software solutions like RADARS (Rapid Automated Data Archiving and Retrieval Software) (Field et al., 2002). In addition to saving the processed data and search results in a relational database, the RADARS system immediately starts database searches for protein identification from raw data files (Field et al., 2002). Peptide mass fingerprinting, also known as peptide mass analysis, peptide sequence tag query, and MS/MS-ion search analysis are the current techniques for analyzing MS data. Real-time identification of protein samples obtained from MALDI-TOF MS processing can be achieved by concurrently searching a sequence database. Online resources offer free software

tools for database searches related to protein identification. These include PeptideSearch (European Molecular Biology Laboratory), MS-FIT (University of California at San Francisco), MOWSE (United Kingdom Human Genome Mapping Project Resource Centre), and MS-TAG (University of California at San Francisco). According to Dančík et al. (1999), algorithms are being developed to assist in deriving the amino acid sequences of peptide fragments from MS/MS data.

Artificial learning models have been developed using protein data from two-dimensional electrophoretic analysis to assist in the classification of tumors into three categories: benign, borderline, and malignant (Alaiya et al., 2000). To that end, statistical methods like hierarchical clustering and partial least squares have been applied. Training sets containing protein profile spectra from the above-discussed technologies are being used to construct algorithms that can cluster and differentiate malignancies from healthy tissue samples. Recently, patients with ovarian cancer and unaffected individuals were distinguished using this method, which had a 100% sensitivity and a 95% specificity (Petricoin et al., 2002). Advances in proteomic technologies are yielding a massive corpus of data that will require handling, processing, and meaningful interpretation. To this end, bioinformatic tools are being developed. These instruments are critical to the identification of precise and sensitive biomarkers in cancer research.

5.8 Proteomic biomarkers in different types of cancers

Proteomics technology for liquid biopsies of cancer. The sources of all bodily fluids—blood, urine, feces, seminal fluid, cervical fluid, ascites, bone marrow, pleural effusion, saliva, cerebrospinal fluid, sputum, lymphatic fluid, and sweat—are described by the inner ring (blue) in the left panel. The outer ring has two colors: red, which represents possible biomarkers of interest, and yellow, which represents nonprotein sources. The red coloration of the latter is further associated with the use of discovery proteomics methods in conjunction with demographic principles (right green panel). These technologies are covered in this study and comprise MS, reverse phase protein arrays, antibody arrays/antigen arrays/beads arrays, proximity extension assay, and aptamer assay. https://doi.org/10.1186/s12943-022-01526-8.

5.9 Renal cell carcinoma

Several research published preliminary results based on tissue proteome analysis with MS, which were then confirmed in human bodily fluids. White et al. (2014) looked into noninvasive diagnostic biomarkers for the identification of clear cell type of renal cell carcinoma (ccRCC). In this investigation, tissue samples (tumor and adjacent nonmalignant tissue) from ccRCC patients (n=40) were analyzed using

liquid chromatography-MS/MS (LC-MS/MS) in conjunction with isobaric tag for relative and absolute quantification (iTRAQ) labeling. In ccRCC patients, a total of 55 proteins were found to have significantly different abundances, 15 of which had increased abundance (iTRAQ ratios of ≥1.5) and 40 of which had decreased abundance (iTRAQ ratios of ≤0.67). Additional investigation using unsupervised hierarchical clustering identified 39 proteins (71%) as "secretory," suggesting that they may have use as ccRCC diagnostic biomarkers. Western blot (WB) was used to select heat shock protein beta-1 (HSP27), neuroblast differentiation-associated protein AHNAK, α-enolase (ENO1), and 10 kDa heat shock protein (HSPE1) for additional verification in the discovery cohort. When ccRCCs were compared to nearby nonmalignant tissues, the expressions of AHNAK, ENO1, and HSP27 were found to be significantly higher (P<.002, P<.01, and P < .01, respectively), while HSPE1 was dramatically downregulated (P < .002). ELISA was used to further validate HSP27 in a small independent group of urine samples (n=17), and the results showed that primary ccRCC patients had considerably higher levels of HSP27 than those without malignancy (P < .05) (White et al., 2014).

5.10 Colorectal cancer

Beginning with its identification in colon cancer tissue, two investigations on colorectal cancer (CRC) were conducted, and the results will be further examined to determine whether they may be used as biomarkers in plasma and serum specimens. Using MALDI-TOF-MS and two-dimensional (2D) differential gel electrophoresis (2D-DIGE), Hamelin et al. (2011) evaluated the levels of protein expression between CRC tissue and the nearby nonmalignant colon mucosa. WB in the tissue samples and ELISA in serum for HSP60 validated the differential expression of glutathione-Stransferase Pi (GST-Pi), ENO1, T-complex protein 1 subunit β (TCP1β), and leukocyte elastase inhibitor (LEI) proteins identified by 2D-DIGE. HSP60 levels were found to be able to distinguish CRC from controls having an area under the curve (AUC) value of 0.70 after sera from 112 patients with CRC and 90 healthy controls were analyzed. An elevated AUC value of 0.77 (P < .001) was obtained when the blood indicators CEA and cancer antigen 19-9 (CA19-9) were combined (Hamelin et al., 2011). Surinova et al. used LC-MS/MS to undertake proteome profiling of micro dissected human primary tumor epithelia in comparison to nearby non-malignant mucosa (n=16 per sample) in order to identify glycoproteins on the cell surface and linked with tumors. 19303 candidates in total were extracted and first quantified using selected reaction monitoring, a focused proteomic technique, in plasma samples from 19 CRC patients. The 303 biomarker candidates underwent additional assessment in two different cohorts. A biomarker signature comprising ceruloplasmin, serum paraoxonase/arylesterase 1 (PON1), serpin peptidase inhibitor clade A (SERPINA3), leucine-rich alpha-2-glycoprotein (LRG1), tissue inhibitor of metalloproteinases 1 (TIMP1), and 34

patients with benign lesions and 100 CRC patients was established in the first cohort. An independent collection of plasma samples from 50 healthy controls, 17 benign lesions, and 202 CRC patients was used to further validate this biomarker signature. The results showed an AUC of 0.84 for the discriminating of CRC patients from the control groups (Surinova et al., 2015).

5.11 Pancreatic cancer

In their analysis of four tissue samples from pancreatic ductal adenocarcinoma (PDAC) and the equivalent non-malignant tissues, Kosanam et al. (2013) found a total of 2190 nonredundant proteins. Of these, 344 proteins were found only in PDAC, and Gene Ontology analysis revealed that 67 of them were secretory and membrane proteins that were released from external receptors. The cellular origin, mRNA expression levels, average label-free quantification values in PDAC tissues, and identification in previously profiled proteomes of malignant pancreatic ascitic fluids were the criteria used to grade the 67 selected proteins. The top four candidates were desmoglein-2 (DSG2), laminin 2 (LAMC2), desmoplakin (DSP), and Golgi membrane protein-1 (GP73). These were subsequently examined using ELISA in serum from 20 patients with benign pancreatic cysts and 20 patients with pancreatic cancer. PDAC patients had considerably higher blood levels of DSG2 and LAMC2 ($P < .05$), but not significantly higher levels of DSP or GP73, according to the research. Additional advantages of LAMC2 may have been discovered through comparison with the CA19-9 standard, which is currently used for PDAC diagnosis (Kosanam et al., 2013).

Tomaino et al. (2011) looked into particular post-translational changes of enolase that cause PDAC patients to produce autoantibodies (Aab).21 Enolase was extracted from PDAC tissues and its expression of six distinct isoforms (ENOA1,2,3,4,5,6) was further investigated using two-dimensional electrophoresis (2DE) and Western blot analysis. The remaining four isoforms (ENOA3,4,5, and 6) showed a threefold increase in PDAC compared to normal pancreatic tissues, while two isoforms were only found in cancer tissue. Circulating Aab against the two enolase isoforms (ENOA1,2) was produced by 62% of PDAC patients, but only 4% of non-PDAC and 9% of serum samples from chronic pancreatitis showed this reactivity. By using LC-MS/MS to examine the phosphorylation of all six ENOA isoforms, it was discovered that serine 419 is the specific phosphorylation site for ENOA1,2 isoforms. A collection of 268 serum samples (120 PDAC serum samples, 40 healthy participants, 50 non-PDAC, 46 patients with chronic pancreatitis, and 12 patients with autoimmune disorders) were used to examine Aab against ENOA1,2. According to Tomaino et al. (2011), the Aab in this investigation allowed for the discrimination of PDAC patients from controls with a sensitivity and specificity of 62% and 97%, respectively.

5.12 Prostate cancer

Pang et al. presented possible biomarkers for 2D-DIGE and MALDI-TOF-MS detection of lymph node metastasis (LNM) originated from prostate cancer (PCa). Six proteins were found to be functionally relevant to cancer metastasis among the 58 identified proteins that were found to express differently in the tissues of PCa patients with LNM compared to patients with benign prostatic hyperplasia (BPH): e-FABP5, mitochondrial methylcrotonoyl-CoA carboxylase beta chain, inorganic pyrophosphatase 2 mitochondrial, ezrin, and stomatin like protein 2 were found to be upregulated, whereas smooth muscle protein (SM22) was found to be downregulated in the cancer tissue. WB and immunohistochemistry (IHC) were used to further corroborate the differential expression of these proteins. Furthermore, ELISA was used to measure the serum levels of e-FABP5 in 20 patients with localized PCa, 20 patients with LNMPCa, and 30 patients with BPH. The results showed that the LNMPCa patients had significantly higher levels of e-FABP5 than the BPH patients ($P < .01$), suggesting that e-FABP5 may be a promising biomarker candidate for the diagnosis of LNM PCa (Pang et al., 2010).

5.13 Esophageal squamous cell carcinoma

Using 2DE and MALDI-TOF-MS, the protein expression profiles of 30 esophageal squamous cell carcinoma (ESCC) tissues and paired adjacent nonmalignant tissues were examined. Heat shock protein 70 (HSP70) and high-mobility group box-1 (HMGB1), two of the 47 unregulated proteins, elicited an autoantibody reaction in ESCC and were more abundant in ESCC than in the nearby nonmalignant tissues. Antibodies against HSP70 showed a substantial increase in ESCC ($P < .01$) in 69 patients with ESCC and 79 healthy individuals; however, there was no significant difference in antibodies against HMGB1 between ESCC and normal controls (Zhang et al., 2011).

In 10 ESCC tissue samples paired with nearby nonmalignant tissue, Hou et al. used a combination of multiple reaction monitoring (MRM) and sequential window acquisition of all theoretical fragment ion mass spectra (SWATH) to identify and validate ESCC-related protein biomarkers. A total of 1758 proteins were quantified by the authors. Of these, 467 proteins showed nominally significant quantitative variations ($P < .05$) between the neighboring nonmalignant tissue and the ESCC, with 260 of them being upregulated and 207 downregulated. Following analysis of the elevated proteins' SWATH MS signals, 116 were chosen and subjected to further MRM assessment in ten matched individual ESCC serum samples obtained before and following tumor removal surgery. Forty-two target proteins out of 116 were found in serum samples. While the remaining proteins did not significantly alter in abundance, 11 proteins had significantly decreased postoperational abundances ($P < .05$) and adequate MRM signals. The abundance of each of these 11 proteins was evaluated further: seven of them had consistently lower levels of abundance in the majority of sera (> 80%) following surgery, while the other four proteins had higher variability (50%–60%) in their changes in abundance, which led to

their exclusion from the final list of biomarkers. Three other proteins—serpin B9 (SERPINB9), dynamin-2 (DNM2), and galectin-3-binding protein (LG3BP)—were identified as novel ESCC-related potential biomarkers (Hou et al., 2015). Of the seven biomarker candidates, glutathione s-transferase omega-1 (GSTO1), histone H4, fibronectin, and thrombospondin-1 (THBS1) were previously described as potential serum biomarkers for ESCC.

5.14 Bladder cancer

Chen et al. profiled proteome alterations in four surgically resected primary bladder cancer tissues and surrounding nonmalignant tissues using a combination of LCM, iTRAQ labeling, and LC-MS/MS. Seven potential biomarkers were identified based on the iTRAQ results: 4F2 cell-surface antigen heavy chain (SLC3A2), carbonic anhydrase 2 (CA2), phosphoglycerate kinase 1 (PGK1), 14-3-3 protein sigma (SFN), stathmin (STMN1), transgelin-2 (TAGLN2), and thioredoxin (TXN). Every one of them was overexpressed in at least three of the four micro-dissected tissue specimens, and IHC was used to confirm this further. When compared to non-cancerous bladder epithelial cells, three of the candidates—SLC3A2, STMN1, and TAGLN2—were discovered to be considerably overexpressed in cancer cells ($P < .001$). Subsequent analysis of urine samples using ELISA showed that bladder cancer patients' urine had significantly higher levels of STMN1 and TAGLN2 (n=104 for STMN1 and n=137 for TAGLN2) than patients' urine (n=48 for STMN1 and n=68 for TAGLN2), with AUC values of 0.67 and 0.70, respectively, when compared to those presenting with hernia (Chen et al., 2015).

5.15 Predictive and prognostic biomarkers for cancer

In order to facilitate early cancer detection and customize treatment choices, biomarkers for cancer diagnosis, prognosis, and prediction are being sought after more and more. In a short research of 18 patients with metastatic bone lesions from lung, prostate, and liver cancer, biomarkers were looked for as an illustration of the possible application of proteomics in cancer detection. After FF tissue samples were analyzed using DIA-MS, the authors found 30 important discriminating proteins. Four important proteins—RFIP1, KRT15, ESYT2, and MAL2—were shown through regression analysis to be significantly differentiable between liver and lung cancer metastases with an AUC > 0.8 (Ku et al., 2020). DIA-MS combined with microdissection was also used to characterize biomarkers in 12 FFPE gastrointestinal tumor samples. CRC samples (n=10) were distinguished from other gastrointestinal tumor samples (n=12) using unsupervised hierarchical clustering analysis (Kim et al., 2018). Microsatellite instability is a significant predictive indicator that shows therapeutic effectiveness. All samples included proteins from the mismatch repair (MMR) pathway, such as MLH1, MSH2, MSH3, MSH5, and MSH6, which were

linked with the samples' genomic status as MMR proficient tumors (Kim et al., 2018). The overlap between clinical markers used for cancer diagnosis and proteomic profiling highlights the potential utility of this technology in replacing manual IHC assessments in cancer diagnosis. These studies used different methodologies with small cohorts and require validation on a larger scale.

It is useful to identify patient subgroups that have varying probabilities of cancer recurrence in order to customize treatment and surveillance. Oropharyngeal squamous cell carcinoma (OPSCC) cases positive for human papillomavirus were analyzed using a hybrid technique combining DDA and DIA-MS to find a recurrence signature (Ho et al., 2021). After analysis of 20 disease-free and recurring FFPE samples, 77 proteins with a substantial log10 fold change were found to be differentially expressed. Using IHC, it was confirmed that several proteins that had not been previously linked to OPSCC—such as LDHB, PFN1, RAD23B, and HINT1—were downregulated in recurrent instances and connected to established carcinogenesis pathways.

Investigating biological determinants of therapy response can be aided by prospective tissue acquisition and window of opportunity research. Prostate biopsies, both fresh and FFPE, were obtained before and 14 days after brachytherapy in a pilot study involving eight patients with prostate cancer. Proteomic profiles were analyzed using DIA-MS (Keam et al., 2018). Following radiation, it was shown that the expression of 49 proteins related to immune activation and wound healing was differentially expressed. Although this study was not designed to identify protein biomarkers of radiotherapy response, it demonstrates a workflow for doing this in future studies.

The response to neoadjuvant trastuzumab-based chemotherapy in HER2-positive breast cancer samples was assessed using a similar experimental design. DNA, RNA, proteome, and phosphoproteome analyses were performed on core needle biopsies that were obtained both before and 48–72 hours after the start of the treatment (Burstein et al., 2015). Significant downregulation of the HER2 protein and alterations in phosphosite signatures for downstream mTOR targets were found in individuals with pathologic full response. There were three categories of resistance mechanisms found. Examples of this included cases in which proteogenomic data suggested that HER2 amplification was not a potent molecular cancer driver, despite clinical diagnostics indicating amplification. This work is an example of how proteomics could enhance current cancer diagnoses and predict therapy responses, even if its main purpose is to generate hypotheses.

5.16 Conclusions

MS technologies have advanced to the point where proteomics in the cancer clinic seems feasible because of several methodological and technological advancements. Proteomics offers the potential to target and customize anti-cancer therapy for each

patient, which is a vital endeavor considering the pivotal role that proteins play in cancer biology. In order to make this a reality, there is a push to advance beyond exploratory landscape studies and take advantage of continuous international cooperation to enhance study design, sample acquisition, and methodology. The adoption of tailored MRM assays for assay validation, multi-omic methods, and enhanced cancer clinician education and engagement are anticipated to facilitate the routine use of these technologies in cancer clinics.

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CHAPTER

Metabolomics: metabolic signatures in cancer biomarkers

6

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6.1 Introduction

The accumulation of unfavorable mutations in genes manifest progression of cancer, resulted cellular reprogramming of metabolism and signal transduction pathways, which in turn uncontrolled cell proliferation, tumor growth, invasion, and metastasis (Loeb & Loeb, 2000). When a normal cell transforms into a cancerous type, it initiates the restructuring of different cellular mechanisms to facilitate its proliferative events. Most important of these reprogramming events include the evasion of cell death, adaptation to a chronically elevated oxidative stress, and metabolic reprogramming to ascertain its energy demands (Sever & Brugge, 2015). Interestingly, the biology of these events is largely governed by the involvement of common cellular components, especially metabolites that nurture cancer cells toward its demand of energy for survival. A key aspect of cancer cell metabolism is the capacity to obtain essential nutrients from surrounding environments by creating tumor microenvironment and use these nutrients to sustain cell growth and generate new biomass to form a tumor (Pavlova & Thompson, 2016). Cancer cells require more nutrients to survive and proliferate than the normal cells. Activation of oncogenes and developing resistance to cell death allow them to adopt altered metabolic and signaling pathways for their growth, cell survival, invasion and metastasis. Mechanistically, tumorigenesis causes an upregulation of the glycolysis and glutaminolysis pathways, which manifest the tricarboxylic acid (TCA) cycle to produce a variety of metabolites, including lipids, amino acids, and nucleotides for supporting their growth and survival (Phan et al., 2014). Three major altered metabolic pathways including carbohydrate, protein, and lipid metabolism enable cancer cells to meet energy demands. Dysregulated catabolism/anabolism of glucose, proteins, and fatty acids generates metabolites and cancer cells utilize them for nutritional supplements for their growth and survival (Hirschey et al., 2015). In addition to that, a crucial characteristic of malignant cells is their ability to adapt persistently elevated level of oxidative stress. Cancer cells generally gather a

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comparatively greater quantity of reactive oxygen species (ROS) than normal cells, seemingly to activate various signaling pathways (Sosa et al., 2013). Elevated oxidative stress in cancer cells is associated with angiogenesis, migration, metabolic reprogramming, and the evasion of cell death (Reuter et al., 2010). Interestingly, ROS appears to be a two-edged sword that acts as both pro-tumorigenic and anti-tumorigenic in a concentration dependent manner (Pan et al., 2009). An elevation in ROS above the threshold of lethal level drags cancer cell towards cell death. Cancer cells cautiously maintain a relatively higher level of oxidative stress without surpassing lethal levels to sustain their growth and proliferation. Thus the generation of ROS via oxidative metabolism and dysregulated anti-oxidant systems contributes to uphold high redox state in cancer cells (Hayes et al., 2020). NADPH oxidase (NOX) is one of the key enzymes, which generates ROS as a primary product and may play an important role in tumorigenesis. In normal physiological conditions, NOX-generated ROS maintains cellular homeostasis, but if generated in an excessive amount leads to oxidative stress and redox imbalance (Waghela et al., 2021). Redox imbalance encompasses the activation of various transcription factors, genes, oncogenes, and proteins of signal transduction pathways, as well as priming the altered metabolism for exaggerated invasion and metastasis of cancer.

Another important feature of cancer cells is their ability to evade the programmed cell death. As a result, the diminishing rate of programmed cell death in cancer cells enables them to self-sustain and proliferate even in the adverse conditions (Sun & Peng, 2009). P53 is a prominent tumor suppressor gene and play vital role as a guardian of the genome machinery, which ensures cell death, when the genetic machinery of a cell is beyond repair (Toufektchan & Toledo, 2018). In the majority of cancers, P53 is mutated or rendered inactive as a result cancer cells allow to accumulate adverse genetic mutations over time. P53 acts as a nodal point that activates a repertoire of apoptotic pathways to ensure a programmed cell death to maintain homeostasis. Mutations in P53 impacts various downstream signaling pathways of cancer cell to uphold their survival and growth (Shu et al., 2007). The loss of the tumor suppressor p53 can trigger the Warburg effect and allows cancer cells to reprogramming of the metabolism for supply of high enegry demand through altered glycolysis pathway. Other oncogenes such as *RAS* and *MYC*, are frequently activated in cancer cells, which contribute genesis of aerobic glycolysis (Cairns et al., 2011). The complexity of cancer biology is still mysterious despite enormous efforts, and research has been done in past many decades. It is interesting to unravel cellular mechanism and unfold advanced approaches for early detection and therapy.

6.2 Reprogramming of metabolism is a key feature for survival of cancer cells

Cancer cells require nutrients, energy, biosynthetic activity, and evasion of the cell death mechanism for continuous proliferation, invasion, and metastasis. It is therefore

not surprising that metabolic activities and programmed survival mechanism in proliferating cells are fundamentally different from normal cells. Metabolic reprogramming, oxidative stress, tumor inflammatory microenvironment and evasion of programmed cell death are key features for the invasiveness of cancer. Recent research revealed that metabolic reprogramming is one of the hallmarks of cancer cells and is applicable to therapeutic approaches (Navarro et al., 2022). Cancer cells utilize energy from various altered pathways including glycolysis, TCA cycle, amino acid metabolism, lipid metabolism, purine metabolism, mitochondrial biogenesis, and other cellular metabolism pathways that are associated with metabolic remodeling in cancer cells (Kim & DeBerardinis, 2019).

6.2.1 Rewiring of energy metabolism and cellular response in cancer

6.2.1.1 Glucose and lactate as a source of energy metabolism

The bizarre behavior of cancer cells to shift toward the less energy-efficient aerobic glycolysis pathway and sidelining an energy efficient oxidative phosphorylation pathway (Warburg effect) for its energy production remains a large enigma in cancer biology. This phenomenon was first observed almost a century ago by Otto Warburg and others in 1923, who observed that tumor cells not only consume more glucose but also generate large amounts of lactate, even when oxygen is present. This observation has confirmed that cancer cells exhibit significant metabolic changes (Liberti & Locasale, 2016). Cancer cells deprived of oxygen utilize glucose for glycolytic metabolism and produce lactate, which is then utilized by cancer cells with sufficient of oxygen (Kim, 2018). Moreover, the restructuring of metabolism is brought about in such a way that it ensures continuous synthesis of amino acids and proteins even in the presence of chronically low levels of ATP inside the cells (Sreedhar & Zhao, 2018). Results from the recent reports suggest the prominent roles of lactate, produced as a result of aerobic glycolysis in cancer progression. In line with these findings, it can be inferred that lactate is a deliberately produced product of cancer cells and is one of the reasons behind the shifting of cell metabolism toward the glycolytic pathway (Dhup et al., 2012; Goodwin et al., 2019). One of the earliest and most notable metabolic changes in cancer cells is their increased glucose consumption. This elevated glucose uptake can be detected using fluorodeoxyglucose-positron emission tomography imaging, which is used for initial cancer staging, monitoring treatment response, and ongoing surveillance (Weber, Schwaiger & Avril, 2000). In the recent past, extensive research was carried out to drive energy sources through mitochondria in cancer cells utilizing lactate to grow and fuel energy to proliferate, and using nutrients from dead cells, as well as altered metabolic pathways for invasion and metastasis (Rabinowitz & Enerback, 2020).

Furthermore, the metabolic reprogramming in cancer cells appears to be far beyond the Warburg effect and shows that p53 regulates metabolism by restricting

glycolysis and facilitating mitochondrial respiration as well as reactive oxygen species generation, which shows a potential link between cancer metabolism, programmed cell death, and other proliferative pathways are closely associated together along with malignant transformation (Matsuura et al., 2016). However, the understandings of these intricate pathways in cancer biology still remain elusive. Earlier studies showed that aerobic glycolysis is linked to the production of **v**ascular **e**ndothelial derived **g**rowth **f**actor, which promotes blood vessel formation (angiogenesis) (Shibuya, 2011). Recent research suggests that abnormal metabolism in cancer cells drives the formation of new blood vessels (Lidonnici et al., 2022). It was well documented that several metabolic enzymes are involved in altered metabolism and cell proliferation of cancer cells such as hexokinase, glucose-6-phosphate isomerase, phosphofructokinase-1 (PFK-1), phosphoglycerate kinase (PGK1), pyruvate kinase (PK), lactate dehydrogenase, pyruvate dehydrogenase complex/pyruvate dehydrogenase kinase, Isocitrate Dehydrogenase, Phosphoenolpyruvate carboxykinase, fructose bisphosphatase, glucose 6 phosphatase, glutaminase, and fatty acid synthase. The upregulation of these metabolic enzymes is associated with many types of cancer (Sreedhar & Zhao, 2018). Indeed, overexpression of these glycolytic enzymes are linked with various altered cellular signaling pathways through the activation of several transcription factors such as p53, Myc, STAT3, HIF1α, and NFκB (Marbaniang & Kma, 2018).

Notably, Pyruvate kinase M2 (PKM2) is a glycolytic enzyme, which is commonly upregulated in many types of human cancers. PKM2 functions to regulate the glycolytic flux and hinders oxidative phosphorylation in cancer cells. PKM2 has been found to play a critical role in gene transcription and cell cycle progression along with metabolism reprogramming (Amin et al., 2019). Cancer cells also have the ability to develop hypoxic micro environment and hijacked all these energy biosynthetic pathways to meet their nutritional energy demand for their growth and survival by mutations, genetic and epigenetic alteration in various genes as well as activation of oncogenes. Cancer cells can build tumor microenvironment by creating hypoxia with subsequent activation of HIF-1α, STAT-3, and NF-kB activation (Rastogi et al., 2023). The activation of HIF-1α mediates transcription of PKM2 to adapt hypoxic conditions and reprogramming of cancer cell metabolism by aerobic glycolysis and lactic acid production (Zheng et al., 2021). It has been recently demonstrated that upregulated expression of PKM2 is associated with activation of NF-kB and pro-inflammatory cytokines in cancer-associated fibroblasts (Gu et al., 2021). In addition to its metabolic functions, nonmetabolic role of PKM2 contributes multiple process including invasion, metastasis, and drug resistance in cancer (Ilhan, 2022). Thus, the metabolic reprogramming of cancer cells has an intricate signaling between metabolism, oxidative stress, programmed cell death, and cell survival signaling as well as developing drug resistance for their growth and survival. The dimer of PKM2 governs glutaminolysis by managing c-myc expression. MYC is a pro-oncogene and play vital role as a transcription factor to regulate transcription and various cellular events including cell growth, differentiation, cell cycle, DNA,

replication, apoptosis and metabolism. Dysregulated expression of MYC has been found in many types of cancer including breast, colorectal, uterine, pancreatic and gastric cancers (Liangwei et al., 2020). Interestingly, the potential involvement of MYC in cancer metabolism was observed, where it regulates important metabolic enzymes, including thymidine kinase (TK), which plays a role in DNA synthesis, and lactate dehydrogenase A, which is involved in the glycolytic pathway (Pusch et al., 1997). MYC can also influence metabolism indirectly through microRNAs (miRNAs). MYC suppresses the transcription of miR-23a and miR-23b, leading to the activation of mitochondrial glutaminase. This, in turn, increases the conversion of glutamine to glutamate and boosts the production of glutamate-derived ATP (via TCA cycle) or glutathione. Consequently, MYC regulates both cellular energy production and levels of ROS (Nagarajan et al., 2016). Oncogenic mutations in *RAS* genes (KRAS, NRAS, and HRAS) and *BRAF* are notably common in various types of cancers and linked with glucose and glutamine metabolism (Hutton et al., 2016).

6.2.1.2 Amino acid as a source of energy metabolism

In addition to glucose, amino acids are pivotal components for cell growth and proliferation. Cancer cells utilize them to sustain cell proliferation and energy requirements using glutamine as a nutrient source. Glutamine is a well-known most prevalent amino acid, which provides nitrogen and carbon source to cancer cells, and providing main source for energy production, macromolecular synthesis, and modulation of various cellular signaling pathways (Coloff et al., 2016). Glutamine acts as prime amino acid to synthesize a wide range of additional amino acids to contribute in TCA cycle. It has been reported that glutamine is the primary driver of the glucose-independent TCA cycle, when glucose is depleted, glutamine-derived fumarate, malate, and citrate greatly rises (Spinelli et al., 2017). Apart from fueling the TCA cycle, glutamine enables fatty acid and nucleotide and biosynthesis, tumor microenvironment as well as activates mTOR signaling in cancer cells (Altman et al., 2016). Recent report revealed that inhibition of glutamine transporters, including SLC1A5, LAT1, and SLC6A14, has shown promising results in preclinical setting and is being actively explored for potential future clinical applications in cancer therapy (Enomoto et al., 2019). Another process that depends on amino acids is purine and pyrimidine-depended nucleotide biosynthesis. Aspartate, glutamine, and glycine provide nitrogen and carbon to purine production in cancer cells (Zhang, Morar & Ealick, 2008). While the pentose phosphate pathway is often recognized as a major contributor to redox balance, a substantial amount of NADPH is also generated by the folate cycle, which is largely fueled by one-carbon units derived from serine (Fan et al., 2014). Amino acid-metabolizing enzymes, including glutaminase 1(GLS1) or GLS2, are responsible for glutaminolysis and glutamine production. Under a hypoxic tumor microenvironment, HIF-1 upregulates the expression of GLS1, which in turn accelerates tumor invasion and migration (Xiang et al., 2019). Thus amino acids serve as an alternative fuel that aids in tumor progression.

6.2.1.3 Lipid as a source of energy metabolism

Lipid metabolism is also involved in rewiring of cancer cells to produce building blocks for membranes, secondary messengers for signal transduction pathways, and energy sources. Cancer cells are known to transform lipid metabolism by accelerating lipogenesis, fatty acid uptake and fatty acid oxidation for accumulation, energy production and plasma membrane synthesis as well as developing tumor microenvironment for favouring cell growth and proliferation (Broadfield et al., 2021). Dysregulated fatty acid metabolism influences composition and saturation of lipid membrane of cancer cells, which in turn modulates tolerance to ROS and influencing their survival (Koundouros & Poulogiannis, 2020). The synthesis of fatty acids and the mevalonate pathway in lipid metabolism are closely linked to the cell growth, differentiation, invasion, and migration of cancer cells (Guo et al., 2020). Fatty acids, phospholipids, and cholesterol are important components of lipid metabolism, which are dysregulated in lipid metabolism of cancer (Fu et al., 2021). Moreover, adipocytes, CAF, and epithelial cells are examples of stromal cells that aid in the reprogramming of cancer cells lipid metabolism. Additionally, lipid metabolites secreted by cancer cells may influence immune cell activation to establish a favorable microenvironment for their growth, invasion and metastasis (Corn et al., 2020). Several possible mechanisms may speculate that dysfunctional adipocytes promote tumor development and progression via supply of lipids to cancer cell for reprogramming the metabolism, promoting inflammation, growth factors, adipokines, and adipocytokines, recruitment of nonadipocyte stromal cells such as inflammatory cells and vascular cells (Font-Burgada et al., 2016). The lipogenesis and chronic low-grade inflammation may induce the secretion of proinflammatory cytokines, chemokines, protease, and protease inhibitors, such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), monocyte chemotactic protein 1 (MCP-1), leptin, and plasminogen activator inhibitor type 1 (PAI-1), which lead to cell proliferation and development of tumor microenvironment and a cancer stem cells (CSCs) niche (van de Vyver, 2023). Hypoxia, glycolysis, and redox imbalance can also affect the homeostasis and regeneration of stem cells. Cancer cells maintain hypoxic condition for reprogramming the metabolism by altered expression of HIF-1α, which is essential for lipid production and storage during hypoxia. HIF-1α and stearoyl-CoA desaturase 1 (SCD1) have a vital role in lipid droplets formation and progression of cancer stemness (Cruz et al., 2020). Thus CSCs and obesity-induced altered metabolism may play a pivotal role in metastasis.

6.2.1.4 Nucleotides as a source of energy metabolism

Nucleotides are proven building block for genetic material and substance for synthesis of DNA and RNA. Nucleotide metabolism plays a pivotal role in the development of tumors and replication of cancer cells (Aird & Zhang, 2015). Cancer cells utilize and synthesize excessive energy and nucleotides for the synthesis of DNA and RNA for cell cycle progression as well as de novo

nucleotide synthesis to support rapid cell proliferation (Wu et al., 2022). Therefore targeting the metabolites from nucleotide metabolism is considered as a potential target for cancer therapy. It has been suggested that Ribose sugars liberated during nucleoside breakdown, and serves as a vital energy source for central carbon metabolism during nutrient deprivation conditions in cancer (Shi et al., 2023). The extended demand of nitrogen is considered one of the key metabolic features of cancer cells, which has been fulfilled by dysregulated nucleotide metabolism in cancer (Pavlova & Thompson, 2016). Several enzymes including carbamoyl phosphate synthetase, aspartyl transcarbamoylase, dihydroorotase (CAD), Inosine monophosphate dehydrogenase (IMPDH), and 5'-monophosphate synthase (GMPS) are kown to be upregulated in cancer to manifest nucleotide metabolism orchestrated by *C-MYC* (Mannava et al., 2008). Numerous cancer patients who are resistant to immunotherapies noticed that the gene responsible for enzyme cytidine deaminase, which is markedly upregulated and involved in the pyrimidine salvage pathway (Scolaro et al., 2024). Thus, compiling evidences suggest that reprogramming of metabolism and their metabolites influences cancer cell growth, invasion, and metastasis. The representative diagram shows the complex networking and association of various molecular players interwind in cancer metabolism (Fig. 6.1).

6.3 Metabolomics in cancer

An early detection or screening of cancer is a major challenge in the field of cancer research and diagnostics. An early detection of cancer using metabolomics may be a beneficial approach for diagnosis, effective treatment, and reducing the mortality. The emergence of advanced analytical technologies and their application using "omics" approaches has provided powerful tools for screening and identifying molecular biomarkers, which could be beneficial for detecting cancer at an earlier stage. Metabolomics is a powerful tool in systems biology, which provides a robust and promising method for discovering biomarkers associated with various diseases such as cancer, diabetes, and metabolic disorders. In practice, metabolomics is defined as the study of small molecule metabolites (up to 1500 Daltons and nonpeptide) within a biological sample. Examination of concentration of metabolites represents molecular phenotype of cancer for prognosis and early detection compared to other techniques. Induction of metabolomics in the field of cancer biology revolutionizes the cancer research for biomarkers development, prognosis, drug design and development, and clinical toxicology for betterment of cancer therapy. Interestingly, advances in system biology more than 2,20,000 metabolites data are available on Human Metabolome Database, those are freely accessible in electronic database (https://hmdb.ca/) (Wishart et al., 2022). Metabolomics approaches were integrated by computational methods for sample and data processing, data collection, and interpretation. Mostly, nuclear magnetic resonance

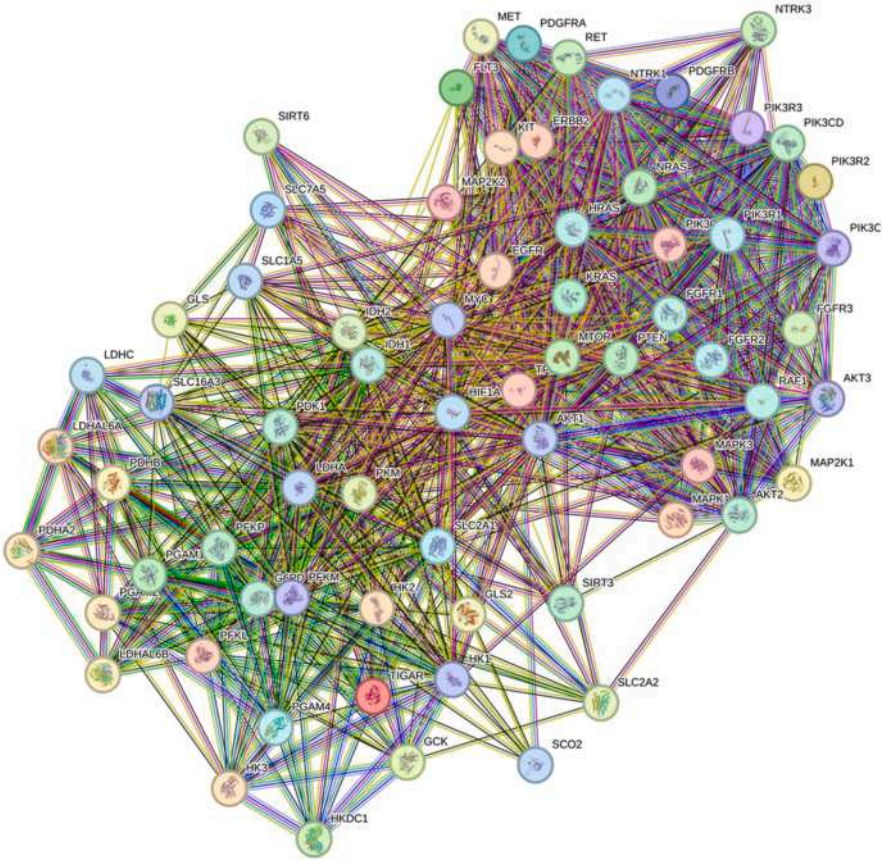

FIGURE 6.1

Central carbon metabolism in cancer. Network nodes represent proteins splice isoforms or posttranslational modifications are collapsed, that is, each node represents all the proteins produced by a single, protein-coding gene locus generated by STRING: functional protein association networks for pathways.

(NMR) spectroscopy and mass spectrometry (MS) were employed in analyses of metabolites. The analysis of metabolites concentration is performed by complex analytical techniques including mass spectrometry (MS), gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE), MS-Ion sources, MS analyzers, and NMR spectroscopy. These sophisticated techniques, coupled with sample preparation, data collection, and data analysis tools, allow to gain a deeper understanding of metabolic processes, identify metabolic signatures associated with diseases like cancer, and facilitate biomarker discovery for diagnostics and therapeutic advancements as shown in Fig. 6.2. Employing metabolic profiling using metabolomic applications and analysis of the data necessitate a comprehensive

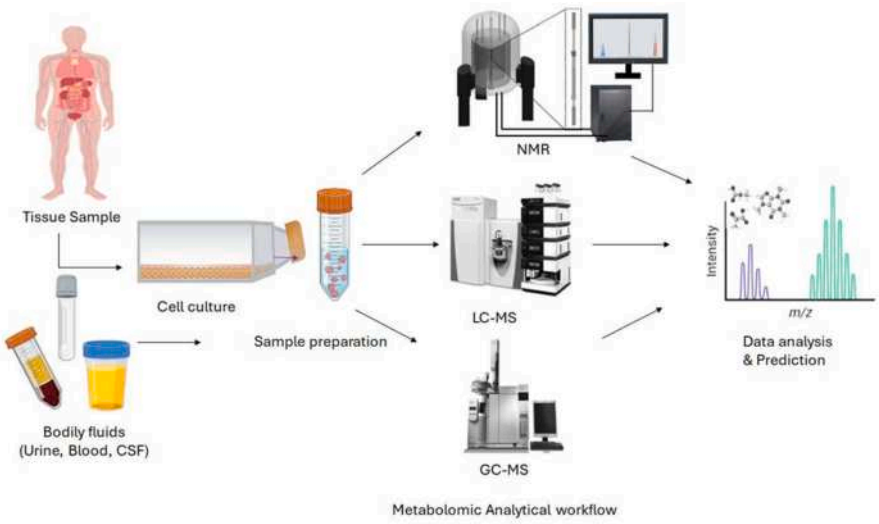

FIGURE 6.2

The representative image shows an analytical workflow of metabolomics.

knowledge of human physiology and computational biology for interpreting data in context to normal and metabolic profiling of tumor sample. However, none of the current analytical platforms can quantify an entire metabolome to clarify the complexities of cancer biology. Recently, numerous metabolites have been recognized through a metabolomic approach for the purpose of developing biomarkers and targeted terapy in cancer.

6.4 Metabolic biomarkers in cancer

Over the past decade, numerous metabolomics studies were performed using NMR and MS techniques for a range of biological samples including blood, urine, plasma, exhaled breath, and tissue to examine metabolite profiles that can distinguish between healthy individuals and cancer patients for early detection of cancer. Here, some studies are discussed, where metabolomics approaches were used to predict metabolites as a biomarker in cancer.

6.4.1 Lung cancer

Mo et al. (2020) had explored the potential biomarkers for lung adenocarcinoma using LC-MS/MS metabolomics approach. In this study, tissue samples were collected from 10 patients of lung adenocarcinoma, including both tumor and non-tumor tissues, as well as from patients with benign lung tumors. The analysis

revealed that adenosine 3′-monophosphate, creatine, glycerol, and 14 additional differential metabolites may be potential biomarkers for diagnosing and prognosis of lung adenocarcinoma (Mo et al., 2020). Zhou et al. (2021) have predicted the modulated levels of elongation factor 1-alpha 2 and spermatogenesis-associated protein could be metastatic biomarkers for lung cancer (Zhou et al., 2021). Recent research indicates that citrulline and phenylalanine are key metabolite markers for non-small-cell lung cancer (NSCLC) using blood sample (Klupczynska et al., 2016). Yu et al. (2017) suggsted that a combination of lipid markers, including ePE(40:4), C(18:2)CE, LPE(18:1), and SM(22:0), can be utilized for the early detection of NSCLC (Yu et al., 2017). High resolution metabolomics profiling of serum sample of lung cancer patients using LC-MS revealed retinol, bisphenol A, and L-proline may be used as a potential diagnostic biomarkers for lung carcinoma (Pamungkas et al., 2016). One of interesting report suggests that metabolomics approach shown strong correlation between osimertinib resistance and the control of amino acid metabolism, HIF-1, and PI3K-Akt signaling pathways (Ma et al., 2020).

6.4.2 Pancreatic cancers

An early detection of pancreatic cancer is challenging because of the lack of reliable biomarker. In recent past, metabolomics approach explored metabolites for diagnosis. Zhao et al. (2023) examined nontargeted metabolomics analysis in tissue samples of 51 pancreatic ductal adenocarcinoma (PDAC) along with healthy control. The study revealed that increased levels of fatty acids and lipids, along with decreased levels of amino acids, were observed in tissue and serum samples of PDAC. Proline, creatine, and palmitic acid were identified as potential biomarkers that could help distinguish PDAC (Zhao et al., 2023). Skubisz et al. (2023) analyzed 154 metabolite from blood serum samples of pancreatic cancer patients. The study revealed that fatty acid metabolite Acylcarnitines are an important marker of energy metabolism found to be at higher concentration in PDAC co-relating with clinical parameters of PDAC patients. The report showed that altered metabolites, specifically acetylcarnitine C2, serotonin, and glycerophospholipid PC aa C34:1, may hold promise as biomarkers for detection of pancreatic tumors (Skubisz et al., 2023). Several amino acid transporters are also found to be highly expressed in pancreatic cancer such as cytoplasmic aspartate transaminase, proline oxidase, and glutamine fructose 6-phosphate amidotransferase-1 during tissue samples of pancreatic cancer patients (Liu et al., 2022). Serum-circulating metabolites, which can be monitored by a noninvasive method using a metabolomic approach, may help in diagnosis and therapy (Zhang et al., 2020).

6.4.3 Prostate cancer

Prostate cancer (PC) can be pointed out by metabolic biomarkers present in the prostatic fluid. Certain metabolites such as myoinositol, polyamine spermine,

citrate, choline, sarcosine, kynurenine, leucine, proline, and uracil were identified using MS techniques (GC-MS, LC-MS) or NMR (Ferro et al., 2019). These metabolites may serve prognosis and predicting its progression, providing insights into the molecular mechanisms underlying prostate tumor growth. Xu et al. (2021) undertook serum metabolic profiling of 39 patients with prostate cancer. In this study, they identified the metabolic biomarker panel for diagnosis performance of PC. According to his research, the metabolites related to phosphatidic pathway were found to be significantly altered in PC patients (Xu et al., 2021). Recent report showed that serum organic metabolites recently identified four potential diagnostic markers: 3-hydroxy-3-methylglutaric acid, citric acid, malic acid, and phenyllactic acid for PC using LC-MS technology (He et al., 2022). Huang et al. (2022) studied the cohort of 1812 cases of PCs for serum metabolome in relation to PC survival using LC-MS/MS platform and found that higher levels of the amino acid glutamate, phospholipid choline, long-chain polyunsaturated fatty acid (n6) arachidonate (20:4n6), glutamyl amino acids gamma-glutamylglutamate, gamma-glutamylglycine, and gamma-glutamylleucine were linked with increased risk of PCs specific mortality, which speculate that higher metabolic score based outcomes may have an elevated risk of PC-specific mortality (Huang et al., 2022).

6.4.4 Breast cancer

Breast cancer (BC) is one of the most profound common cancers in the women worldwide and causes cancer-related death. In recent study plasma metabolomic profiling of 55 BC patients along with healthy controls was conducted using ultra-HPLC coupled with quadrupole time-of-flight MS (UHPLC/Q-TOF-MS) revealed significant changes in amino acid esters (*N*-stearoyl tryptophan, L-arginine ethyl ester), dipeptides (ile-ser, met-his), nitrogenous bases (uracil derivatives), lipid metabolism-derived molecules (caproleic acid) (Da Cunha et al., 2022).

6.4.5 Ovarian cancer

Many metabolomics markers related to ovarian cancer (OC) have been identified through metabolomic studies, allowing for their use in diagnostic and therapeutic applications. Study undertaken by Tang and his colleagues found 24 lipid metabolites that were significantly changed in the pelvic fluid of ovarian cancer patients. Palmitoylcarnitine and lipoamide were the most potential one, some of the lipid metabolites were notably associated with clinical stages showing diagnostic value for early detection and prognosis of OC (Tang et al., 2024). For Epithilial Ovarian Cancers (EOC) distinct amino acids and organic acids such as methionine, asparagine, glutamine, glutamic acid and glycolic acid were identified as potential biomarkers. The training set's metabolic network analysis revealed the critical roles that glutamate, aspartate, and alanine metabolism, as well as the metabolism of D-glutamine and D-glutamate, play in the pathophysiology of EOC (Wang et al., 2021).

While some metabolites, such as L-threonine, l-pyroglutamic acid, benzoic acid, creatinine, and pentadecanol metabolites were determined as prominent biomarkers (Eroglu et al., 2022). In the present study machine learning approach was employed to identify OC related potential biomarkers and revealed that pathways for Glycolysis/Glyconeogenesis, Nicotinate and Necotinamide metabolism, biosynthesis of valine, leucine, and isoleucine, and aminoacyl-tRNA biosynthesis, are involved in the metabolites generation in OC (Yao, Tsigelny, Kesari & Kouznetsova, 2023). On the other hand, Cao et al. (2024) demonstrated inducing and protective effect of Deoxycholic acid glucuronide levels and 12-hydroxyeicosatetraenoate levels on ovarian cancer in serum samples (Cao et al., 2024). Each of these investigations makes abundantly evident the significance and wide-ranging use of metabolomics to monitor the precise alterations in patients' metabolites for detection of cancers before progressing to final stage. However, further studies are necessary to comprehend the roles and patterns of expression of these metabolites, which may eventually can be used as novel biomarkers for detection and targeted therapy in cancer. In the recent past, metabolites those were analyzed in cancer patient samples using metabolomic approaches shown in Table 6.1.

6.5 Metabolomics as a therapeutic target in cancer

In the recent past new therapies aimed at cancer metabolism and emerged due to an increasing interest on metabolomics. These metabolism-based treatments aim to target particular metabolic pathways implicated in the development and progression of tumors. These medications either deliver a metabolic product that modifies tumor metabolism or inhibits the target enzyme implicated in the pathway (Suri, Kaur, Carbone & Shinde, 2023). Metabolomics can help medical professionals in the recognition of the intricate nature of cancer and the identification of potential new targets by elucidating pathways. For instance, metabolomics investigations revealed reduced levels of specific metabolites in triple-negative BC patients with BRCA1 mutations compared to those without BRCA1 mutations. These metabolites might serve as possible markers linked to mutations in the BRCA1 gene (Roig et al., 2017).

Chemicals known as "antimetabolites" have been used in the earliest cancer treatments (Elion et al., 1954). Since they interrupted the normal metabolism of pathways and exhibited chemical similarities with endogenous metabolites within different pathways, they were known as antimetabolites. The antimetabolites methotrexate, cytarabine, and 5-fluorouracil targeted late-stage DNA synthesis. Targeting altered cancer metabolism as a therapeutic approach during therapy has developed a significant interest (Vander Heiden, 2011). Moreover, many anti-cancer drugs not only targeted nucleotide and DNA synthesis, but also influences other metabolic processes and adverse side effects. More recently, research has

Table 6.1 Metabolites in cancer and their detection process.

Metabolites	Cancer type	Detection technique	Reference
D-2-hydroxyglutarate (D-2HG)	Gliomas	magnetic resonance spectroscopy (MRS)	Andronesi et al. (2012)
2-Hydroxyglutarate (2-HG)	AML, breast cancer, renal cancer, intrahepatic cholangiocarcinoma, papillary thyroid carcinoma		Wang et al. (2013)
pyruvate dehydrogenase		magnetic resonance spectroscopy (^{13}C MRS)	Cheshkov et al. (2017)
GlycA (glycoproteins containing *N*-acetylglucosamine and *N*-acetylgalactosamine portion), and GlycB (glycoproteins containing *N*-acetylneuraminic acid portion)	Metastatic prostate cancer	NMR	Cacciatore et al. (2021)
Elevated levels of glucose, mannose, pyruvate and 3-Hydroxybutyrate in plasma	Papillary thyroid microcarcinoma	NMR	Lu et al. (2016)
Creatine, inosine, beta-sitosterol, sphinganine and glycocholic acid	Pancreatic cancer	LC-MS	Luo et al. (2020)
1-methyl adenosine (1-MA), 1-methylguanosine (1-MG) and 8-hydroxy-2′-deoxyguanosine (8-OHdG)	Breast cancer	GC-MS	Omran et al. (2020)
L-glycine, phosphoric acid, isocitric acid and inositol	Lung cancer	GC-MS	Callejon-Leblic et al. (2019)

concentrated on creating substances that deplete amino acids and target key metabolic pathways, such as glycolysis, the TCA cycle, and lipogenesis, that are aberrantly controlled in cancer cells. While some of these treatments are now being evaluated in clinical trials, and many are still in the preclinical phases. It is important to emphasize that treatments that target metabolic enzymes can effectively slow down the growth of various solid tumor types in preclinical models; however, tumor

regression is not always observed, suggesting that some of these agents may be best used as maintenance therapy or in combination with other agents (Schmidt et al., 2021). In the battle against cancer, metabolomics has evolved as a powerful tool that makes it possible to identify changes in metabolism and develop personalized therapies precisely (Danzi et al., 2023). Using targeted metabolomic approach it may possible an early detection of cancer and restore metabolic homeostasis by targeting specific metabolic pathways (O'Connell et al., 2021).

6.6 Conclusion

Cancer is a disease of complexity, and it is the leading cause of death worldwide. Oncogenic transformation manifests cell proliferation, inhibition of programmed cell death, and altered cellular processes and responses. The reprogramming of cancer cells enables them to continue growing and surviving while also defending against drugs by acquiring resistance. Cancer cells adopt altered metabolism, cellular signaling, and response by crafting alternate pathways to meet energy demand and supply building blocks for cellular components to enable continuous cell proliferation, invasion, and metastasis. During progression, metabolic phenotypes are produced by reprogramming cellular metabolism, and they can be used as biomarkers of treatment as well as for patient selection in clinical trials and an early cancer detection. Since past several decades cancer research was most demanding in the field of biological sciences, and although considerable advancements and findings have been made, but there remains a great deal to understand regarding the mechanisms and identification of cancer. A comprehensive study of metabolites in different types of cancer may hold promising value for biomarker development for early detection. Therefore by exploring the cancer metabolome, metabolomics may provide valuable insights into the biological functioning and phenotypic changes in the profiling of different types of cancer. Extensive research is needed to manifest comprehensive metabolic profiling and integration with genomics, proteomics, and transcriptomics to enable the discovery of potentially new biomarkers for the early detection of cancer and therapy.

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CHAPTER

Biomarker validation: challenges and regulatory perspectives

7

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7.1 Introduction

Cancer is defined as a group of diseases that occur as a consequence of abnormal cell growth and have the potential ability to spread and invade in different body parts. Cancer can begin in any part of the body; it grows with abnormal and damaged cells in trillions of numbers, leading to the formation of tumors as tissue lumps (Ou et al., 2021). Tumors could be cancerous or noncancerous. As per the WHO, cancer is one of the leading causes of death worldwide, causing approximately 10 million deaths in 2020—one in every six deaths is due to cancer. Breast, prostate, colon, rectum, and lung cancers are the most common cancers (Fig. 7.1) Early-stage detection of cancer can be cured and effectively treated (no date).

As defined by the National Cancer Institute, the biomarker is "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease." Biomarkers act by biological phenomena at the molecular level and are utilized in healthcare, as they provide insights, with change in the process (as in metabolite, protein, or nucleic acid) reflecting the underlying malignancy progressive stage (Henry & Hayes, 2012; Wu & Qu, 2015). Biomarker tests in tumors are mainly used to identify whether the patients are responding to treatment, with treatment plans adjusted based on the response. It helps in patient management based on tumor biomarker tests or assays. Biomarkers are available in tremendous varieties such as antibodies, peptides, proteins (receptors or enzymes), and nucleic acid (non-coding RNA or microRNA). They can be assessed in blood, plasma, or serum, as well as secretions or excretions (such as urine, stool, nipple discharge, or sputum). Biomarkers can also be evaluated through tissue samples, special imaging, and biopsy for further analysis (Mäbert et al., 2014; Mattes & Goodsaid, 2018).

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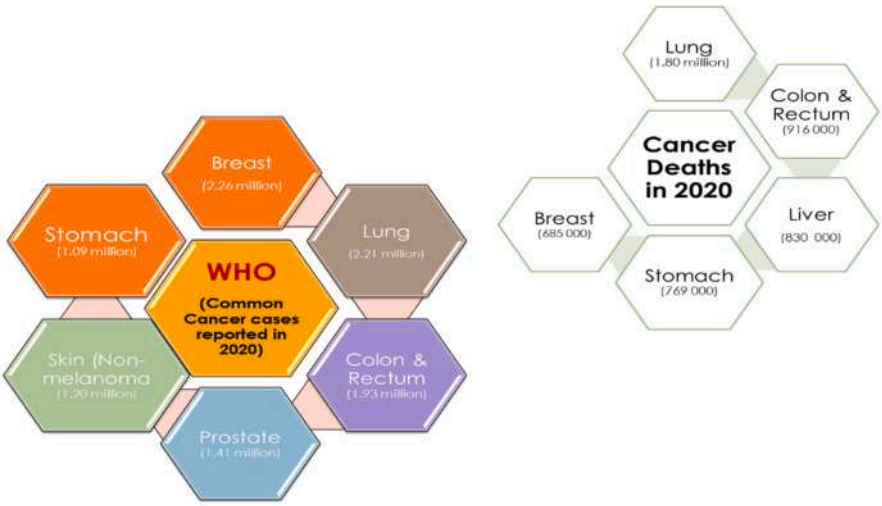

FIGURE 7.1

Illustration of various types of cancer and estimated patients, WHO 2020.

In cancer, biomarkers have potential applications, they are helpful in prognosis determination, differential diagnosis, screening, treatment response prediction, and monitoring disease progression (Hayes, 2015; Zhou et al., 2024). Cancer biomarkers play a key role in disease at all stages, in tumor heterogeneity based on sensitivity, and implementing biomarkers in aggressive cancer is helpful in early-stage detection. In oncology precision medicine has been achieved with transition by early detection, achieving comprehensive cancer evaluation based on utilizing molecular tools and data integration. Proteomic and genomic cancer biomarkers have led to dramatic growth in identifying potential markers and incorporating them into routine clinical and medical practices (Jimenez & Verheul, 2014; Polanski & Anderson, 2006).

Cancer biomarker validation is a crucial process to ensure the reliability and application of the biomarker in clinical procedures and its study (FDA_Biomarker & pdf, no date). There are many steps involved in the procedures of biomarker validation, such as discovery, analytical and clinical validation, and clinical utility (Hristova & Chan, 2019) (Fig. 7.2) During the discovery phase, potential biomarkers are initially identified based on research utilizing high-throughput techniques, based on omics approaches like proteomics, genomics, and metabolomics. During the validation studies, the properties of biomarkers are quantified accurately and their reliability across the various samples and at different settings are analyzed to be validated. Validation parameters include the accuracy, precision, and assay reproducibility. These parameters are specifically studied to ensure that biomarkers can be appropriately detected in biological matrices such as blood, body fluids, tissue, etc. Clinical validation is performed to ensure the correlation of biomarkers with clinical

FIGURE 7.2

Various levels are involved in the development of a biomarker.

outcomes. Studies are performed with research techniques of retrospective, as well as prospective and, obtained data utilized for interpretation, identifying the biomarker-specified clinical end-points based on the disease progress, survival, and treatment response. The clinical utility process provides information on the biomarker evaluation parameter, based on the patient's clinical condition improvise and outcomes. Biomarker pathophysiology and pathways are studied to understand the modes of action, their influence on the selected treatment regimen, and their impact on health outcomes (Goossens et al., 2015; Mäbert et al., 2014).

Cancer biomarker application in clinical practice requires regulatory approval and they have to go through rigorous processes. Various regulatory processes involved are preclinical studies, clinical trials, regulatory submission, approvals, and post-marketing surveillance (Mattes & Goodsaid, 2018; Zhou et al., 2024). During the preclinical studies, the initial study's main objectives are to discover that a biomarker is biologically acceptable, plausible, and accurately measurable in the body. Biomarker clinical trials are conducted in multiple phases to assess the efficacy, safety, and utilization in large and diverse groups of patient populations. Approval from regulatory bodies requires the submission of data related to the analytical study and clinical validation studies (Hayes, 2015; Quezada et al., 2017), which is obtained on the interpretation of biomarker application and usage, along with the therapeutic and diagnostic data. After the biomarker is approved for clinical practice usage, it will be continuously monitored throughout the period it is available in the market to ensure the safety and efficacy of the biomarker. During the post-market surveillance, data collected from the broad patient population group are continuously analyzed to ensure biomarkers' efficacy and therapeutic application (Henry & Hayes, 2012; Masucci et al., 2016).

7.2 Validation of cancer biomarkers

7.2.1 Analytical validation

Identifying and validating biomarkers is a time-consuming and critical phase since the identification and quantification of cancer biomarkers require highly sensitive assays. Another factor that must be considered carefully is the choice of assay platforms because different biomarkers have different characteristics, meaning that various technologies must be used for the detection to obtain the desired sensitivity, specificity, and throughput. Among the tools that are used are Enzyme-Linked Immunosorbent Assay (ELISA), quantitative Polymerase Chain Reaction (qPCR), next-generation sequencing (NGS), and Immunohistochemistry (IHC) (Masucci et al., 2016; Pal et al., 2022). For instance, the molecular diagnostics highlighted in the research by Chau et al. show how these platforms are hinged on the synergy between them to ensure primarily accurate and precise biomarkers identification in numerous clinical uses (Chau et al., 2008). These platforms are chosen depending on the needs of the biomarker in question; for instance, qPCR is preferred for its ability to detect genetic mutations with high sensitivity, whereas NGS is used for its capacity to offer broad genomic data. However, these technologies must be through the validation of the technology to achieve consistent and accurate results across the various laboratories; this is a significant step that is needed before the technology is put into practice (Barker, 2003; Goossens et al., 2015).

Reproducibility is an essential facet in assay development since these inconsistencies greatly contribute to differences in the clinical management of a patient. The concept of method validation, under different conditions, qualifies the assay's ability to perform uniformly in other laboratories or diverse conditions. For example, in a study by Wang et al., the inter-lab reproducibility of diagnostic tests was assessed by conducting quality control measures that eliminate variability (Wang, 2015). These principles apply to every aspect of the assay, including sample collection and preparation, analysis, and report generation, thus guaranteeing the assay's performance. Standardization is mainly useful when many centers are involved, for instance, in large-scale clinical trials. These protocols establishment has reduces disparities that might occur as a result of variation in practices from one laboratory to the other, thereby increasing the credibility of the biomarker assay.

Sensitivity and specificity are important factors that define the practicability of an assay in a clinical laboratory. Specificity concerns the capacity of the test to recognize true positive samples, which includes differentiating one copy of ctDNA in blood, which is so significant in the early diagnosis of cancer (Zhu et al., 2011). Studies show how critical it is to have high sensitivity in the assays because even at very-low concentrations of biomarkers such as ctDNA, valuable information regarding the presence and development of cancer is obtainable. For example, in establishing prostate cancer diagnosis, assay sensitivity of epigenetic markers detection such as GSTP1 methylation becomes crucial in the differentiation of malignant tissues from nonmalignant ones, hence enhancing the effectiveness of

early diagnosis and treatment (Hernández & Thompson, 2004). On the other hand, specificity is the ability of a given assay to give a negative result to a truly negative sample. That is, it does not give a positive result to a negative sample. High specificity is important in that patients who do not have the disease in question should not be told that they do, as this would cause more suffering and expenditure on ineffective treatment. The notion is that these two parameters of sensitivity and specificity must be optimized but should be done so in a way that will not limit the clinical application of the assay. For instance, in breast cancer diagnostics, there are tests such as Oncotype DX that have been developed to give a high specificity in terms of an individual's likelihood of developing recurrent disease, thus helping in choosing the ideal treatment regimen (Curtit et al., 2017).

The other concern is the robustness of the assay procedure, which deals with the stability of the procedure in different situations, including the type of sample, temperature, relative humidity, and many other factors. The robustness of the assay allows the use of the assay across different clinical settings without any interference in assay performance. ELISA and IHC are some of the conventional clinical diagnostic techniques that help to identify cancer biomarkers, and these techniques need to exhibit stability when used in different laboratories and patients. This entails, for instance, being able to perform well in consistency even with differences in sample quality, storage time, or procedures. For instance, speaking of CTCs (circulating tumor cells), the stability of assays like CellSearch, which enables detecting and counting CTC in various types of cancer, is significant for using these biomarkers in tracking changes in cancer progression and reaction to therapy (Andree et al., 2016). Maintaining robustness will allow the researchers to be confident the assay will provide consistent outputs, regardless of any variability from outside interference, thereby increasing the credibility of biomarker-based diagnostics in day-to-day use.

Later, in conclusion, one can state that the process of validation of cancer biomarkers comprises the following steps: assay development, sensitivity, specificity, and robustness, which are necessary for further usage of biomarkers in practice and can be viewed in the Venn diagram provided in Fig. 7.3 The process is rather challenging, and proper validation is of the utmost importance when it comes to applying biomarkers as diagnostic or therapeutic targets in clinical practice. Such an approach guarantees that developed assays are not only valid from the point of view of a scientific paradigm but also sound and effective instruments for cancer diagnosis, prognosis, and individualized treatment assessment. As the biomarkers are proven to be essential in personalized medicine, the process of proving the authenticity of these molecules becomes essential.

7.2.2 Clinical validation

The biological confirmation of cancer biomarkers is a complex and often step-by-step process of verifying and approving these biomarkers before they are commonly used

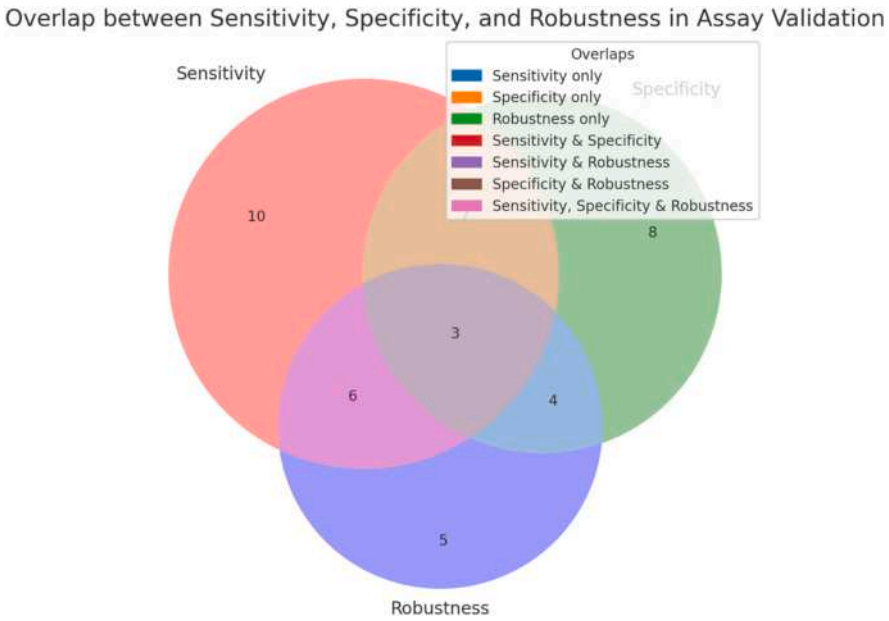

FIGURE 7.3

A Venn Diagram representing the overlaps between sensitivity, specificity, and robustness in assay validation (Andree et al., 2016; Barker, 2003; Chau et al., 2008; Curtit et al., 2017; Goossens et al., 2015; Hernández & Thompson, 2004; Masucci et al., 2016; Wang, 2015; Zhu et al., 2011).

in clinical practice. All of this takes place in a number of stages of clinical trials, important in different stages of progression from the time the drug is discovered until it is widely prescribed (Mordente et al., 2015). Phase I Clinical Trials represent the first step of this process in which little, if any, efficacy is investigated, and the main goal is to evaluate the toxicity of new therapies and monitor the biomarkers. This phase is important as it defines the biomarker's behavior in the framework of the treatment program and its safety and measurability in patients. For instance, the recognition of colorectal cancer biomarkers by Yeonjoo Jung and companions consisted of evaluating the levels of candidate genes, including ECT2, ETV4, and DDX21, in tumor and normal tissues. Such early studies are crucial for the assessment of the practical potential of these biomarkers for subsequent study in larger patient cohorts (Jung et al., 2011). Subsequent to the safety evaluations conducted in Phase I, Phase II Biomarker Trials move a step further by conducting mid-sized trials that address the biomarker's effectiveness and its potential predictive capability as to therapeutic outcomes. This phase also involves the identification of the cut-off values for biomarkers—these being the levels of the biomarkers that are of clinical importance. For example, in the context of HCC (hepatocellular carcinoma),

the content of biomarkers such as AFP (alpha-fetoprotein) and FGF19 (fibroblast growth factor 19) has been examined during Phase II (Piratvisuth et al., 2023). Nault and Villanueva (2021) as well as other researchers used these biomarkers to find out whether they were useful in assessing patients' responses to therapies such as ramucirumab and FGFR inhibitors. The results of these trials are important in defining the biomarker, defining its possibilities, and setting the stage for its application in determining the course of further therapy. There is no doubt that Phase III Clinical Trials are the longest duration of clinical validation. Such large-sample investigations are designed to validate the biomarker's applicability across presumably diverse patient populations; this is done to guarantee that the biomarker can consistently and positively impact patients' well-being when it is incorporated into treatment plans. For instance, within Cholangiocarcinoma (CCA), Phase III trials helped determine the applicability of fibroblast growth factor recpetors (FGFR) inhibitors in the treatment of FGFR2 fusion-positive patients and Isocitrate Dehydrogenase 1 (IDH1) inhibitors in IDH1 mutation-positive patients. These trials, conducted by researchers including Reinhard Drummer, Jan C. Brase, et al., are meant to establish that this biomarker not only has accuracy, as established by earlier studies, but also helps improve patients' outcomes in a typical clinical practice—-which is essential for its approval for routine clinical use (Dummer et al., 2020).

In addition to the formal phases of clinical trials, the validation process normally has an integration system, where bioinformatics analysis and validation are intermingled. This approach was illustrated in the studies by Jung et al., in which bioinformatics utilities were employed in order to scan databases for potential genes. These candidate biomarkers were then given through other clinical trials to assess the validity of the expression as well as the association with clinical results (Jung et al., 2011). Such a comprehensive strategy guarantees that biomarkers receive rigorous scrutiny that puts them on the list of dependable and efficient tools in clinical practice. Therefore the test validation of cancer biomarkers is a well-coordinated sequential process that is significant to warrant the safety, efficiency, and applicability of these biomarkers in clinical practice. As the trials of these clinical phases move from Phase 1 and Phase 2 evaluations of safety and efficacy studies to Phase 3, it becomes more evident that these biomarkers are good scientific markers and also help in improving the accuracy and efficiency of cancer treatment in actual practice. Such a meticulous process, with the help of professional experts like Jung, Nault, and Villanueva, is vital in converting scientific research into lifesaving tools. The various validation stages and the key biomarkers used in the above literature are summarized in Table 7.1.

7.2.3 Biomarker performance metrics

Therefore it is crucial to comprehend and assess the biomarkers' performance regarding diagnostic and prognostic assessment in the disorders. Several performance measures are employed to evaluate the degree to which a biomarker

Table 7.1 Various clinical validation stages in determining cancer biomarker efficacy and safety (Dummer et al., 2020; Jung et al., 2011; Mordente et al., 2015; Nault & Villanueva, 2021; Piratvisuth et al., 2023).

Validation stage	Objective	Biomarkers example	Key findings
Phase I: Safety & feasibility	Assess the safety, toxicity, and initial biomarker behavior within a small patient cohort.	ECT2, ETV4, DDX21	These early studies help determine whether biomarkers are measurable, safe, and potentially effective, setting a foundation for further trials.
Phase II: Efficacy & cut-off determination	Evaluate the effectiveness of the biomarker in predicting clinical outcomes and establish clinically	AFP, FGF19 (in HCC context)	This phase focuses on determining how well the biomarker predicts therapeutic outcomes, crucial for refining its use in clinical decision-making.
Phase III: Large-scale validation	Confirm the biomarker's utility in diverse patient populations and its ability to improve clinical outcomes.	FGFR inhibitors (FGFR2 fusion-positive), IDH1 inhibitors.	Extensive trials validate the biomarker's effectiveness in real-world settings, ensuring it consistently improves patient care across different demographics.

discriminates between those with and without a disease, thus aiding clinicians and other healthcare practitioners in their decision-making process. From all of these, receiver operating characteristic (ROC) curves, PPV (positive predictive value), and NPV (negative predictive value) are important measures that define the performance of the biomarker (Agraz et al., 2024; Gogtay & Thatte, 2017). ROC curves are the gold standard for assessing the diagnostic performance of biomarkers. These curves show the relationship between the sensitivity (true-positive rate) and false-positive rate (1-specificity) as a function of the threshold settings. They can give a clear and quantitative indication of how well a particular test performs on the diseased and non-diseased populations (Oldenhuis et al., 2008). The ROC curve discriminant index values are informative, particularly the area under the curve, which is approximately equal to 1. In summary, the reader can classify the primary outcomes of the proposed method as follows: sensitivity = 0, specificity = 0, and, therefore, the perfect score of 0 reflects the model's excellent discriminatory ability (English et al., 2016). For instance, during the development and validation of GAAD (Gender, Age, AFP, Des-Gamma Carboxy-Prothrombin) for the identification of early HCC, the ROC analysis indicated an AUC (area under curve) of 90 (Piratvisuth et al., 2023). This high AUC further confirmed that the proposed

GAAD algorithm can accurately classify early-stage HCC from non-HCC cases. It outperformed AFP and PIVKA-II (Protein Induced by Vitamin K Absence or Antagonist-II) as biomarkers. A high AUC indicates that the GAAD algorithm should have a good ability to eliminate the interference between true-positive rates and false-positive rates, making it ideal for early diagnosis.

Another measure is the PPV, which determines the likelihood that patients with a positive test result actually have the disease (Monaghan et al., 2021). PPV increases as the prevalence of the disease in the population being tested increases. The complementary to PPV is NPV. For instance, in the context of the GAAD algorithm analysis, the PPV estimation employed SEER (Surveillance, Epidemiology, and End Results) cancer incidence rates to control for population-related characteristics. The authors discovered that the adjusted PPV was 44.4% for patients aged 50–79 years, the algorithm is therefore highly effective in establishing true diagnoses of HCC among the positives (Piratvisuth et al., 2023; Shapley et al., 2010). This level of PPV is especially crucial in a clinical context where false-positive results could cause distress and additional probing diagnostic tests to patients. Along with the PPV, the NPV is used and denotes the likelihood of the absence of the disease in subjects without a positive test result. NPV is, therefore, particularly important in conditions where a definitive exclusion is desired in a screening program. A high NPV means that people who undergo the test and get a negative result can be quite confident that they do not have the disease, so there is no need for follow-up tests or ongoing surveillance (Yotsukura and Mamitsuka, 2015). In the case of the GAAD algorithm, the expected NPV was computed to be 99%. Specifically, its accuracy could be estimated at 4%, which testifies to its efficiency in excluding HCC in the context of surveillance in patients. This high NPV is useful, especially considering the concomitant reduction in the rate of unnecessary follow-up tests and interventions. Evaluating a biomarker model using ROC curves, PPV, and NPV provides a good overview of its clinical applicability. Analyzing such biomarkers can help researchers and clinicians find their advantages and drawbacks, which will lead to more precise diagnostics, prognosis, and treatment (Simon, 2010; Taylor et al., 2008). The results indicate that the GAAD algorithm has achieved excellent results in these metrics are illustrative of how advanced biomarkers can improve disease detection and patient care, Table 7.1 is provided to view the key biomarkers and their examples using the GAAD algorithm (Table 7.2).

7.2.4 Statistical considerations

The actual development of biomarker discovery and validations, design, and conduct studies should also include good statistically validated methods for acquiring reliable and valid results. These statistical considerations may consist of selecting the right sample size, sample power adequacy, and multiple testing corrections to reduce the rates of false error (Polley et al., 2013). Determination of an appropriate sample is a very important aspect in the planning of the study, particularly in biomarker studies,

Table 7.2 Key biomarker performance metrics and their examples using the GAAD (Gender, Age, Alpha-fetoprotein, Des-Gamma-Carboxy-Prothrombin) algorithm (Agraz et al., 2024; English et al., 2016; Gogtay & Thatte, 2017; Monaghan et al., 2021; Oldenhuis et al., 2008; Shapley et al., 2010; Simon, 2010; Taylor et al., 2008; Yotsukura & Mamitsuka, 2015; Huang et al., 2023).

Metric	Definition	Example/use case	Key insights
ROC curves	ROC (receiver operating characteristic) curves plot the true positive rate (sensitivity) against the false positive rate (1-specificity) to assess the diagnostic accuracy.	Used to determine the effectiveness of the GAAD algorithm for early HCC detection, where the AUC (area under curve) was 90%, indicating high diagnostic accuracy.	A high AUC suggests strong discriminatory ability, making the biomarker effective for early diagnosis or screening purposes.
Positive predictive value (PPV)	The probability that patients with a positive test result actually have the disease.	In the GAAD algorithm analysis, PPV was adjusted to 44.4% for patients aged 50–79 years, making it effective in confirming true cases of HCC among positive test results.	High PPV is crucial for minimizing false positives, ensuring that patients identified as positive truly have the disease, which is important for targeted interventions.
Negative predictive value (NPV)	The probability that patients with a negative test result which do not have the disease.	The GAAD algorithm achieved a high NPV of 99.4%, indicating its efficiency in ruling out HCC in patients, reducing unnecessary follow-ups.	High NPV is essential in screening contexts, ensuring that those who test negative can confidently be excluded from having the disease, reducing the burden on healthcare systems

since the aim of studies involves coming up with a statistic that has a specific margin of error that is acceptable for generalization to the entire population if the need arises. The sample size needs to be big enough in order to be able to pick the signal, if there is any. For instance, in biomarker discovery studies using proteomics platforms, small sample sizes result in underpowered studies that do not define worthwhile biomarker differences between the tested groups (Skates et al., 2013). To have enough power, statistical models use sampling by providing hypotheses of various parameters and estimating how many samples are needed due to the size of the measured biomarker's effect, variability, etc., and number of comparisons.

Statistical power, instead, relates to the prospects of the convicted null hypothesis when, in-fact is fake or their "ability to detect an effect." A low-power

study is liable to commit a Type II error whereby a significant effect goes undetected due to spurious factors such as inadequate sample size (Ray et al., 2010; Weir & Walley, 2006). Another important aspect of biomarker studies relates to power, especially in the case of significant variability of biological material or low concentrations of the target proteins. For instance, in the process of designing a study, a typical power will be fixed at 80%–90%, which literally means that the study will have an 80%–90% chance of detecting an existent effect given a certain scenario provided in the study. Power calculations make sense when they draw on simulation studies to express the worth of factors that influence power so that researchers can modify their study design before they actually start the process. In clinical practice, when patient data are examined for several potential biomarkers at the same time, the danger of having a Type I error or a false-positive result is considerably higher. In order to avoid this risk, corrections to multiple testing are done. The simplest approach is the Bonferroni correction whereby the original alpha level is adjusted according to the number of comparisons being done (Shi et al., 2012).

Nevertheless, the Bonferroni correction is said to be quite over-conservative, particularly where the tests are related. Because of this, it can lead to a low power level in the study. In response to this, versions of the Bonferroni correction are sometimes employed at least as a method of at least attempting to control the overall error rate or other more efficient methods, including the Holm procedure, intraclass coefficient correlation (ICC), or resampling techniques. These methods seek to achieve two objectives; ensuring that the probability of obtaining false-positive results is low while at the same time keeping the probability of a type II error as high as is reasonable. For example, in a study involving multiple correlated endpoints, the application of an ICC-based correction factor was shown to effectively control the Familywise error rate (Cox et al., 1996). A flow chart is provided in Fig. 7.4 to summarize the statistical considerations.

Therefore statistical consideration in biomarker selection is crucial in biomarker studies. In other words, the main benefits of statistical power and sample size

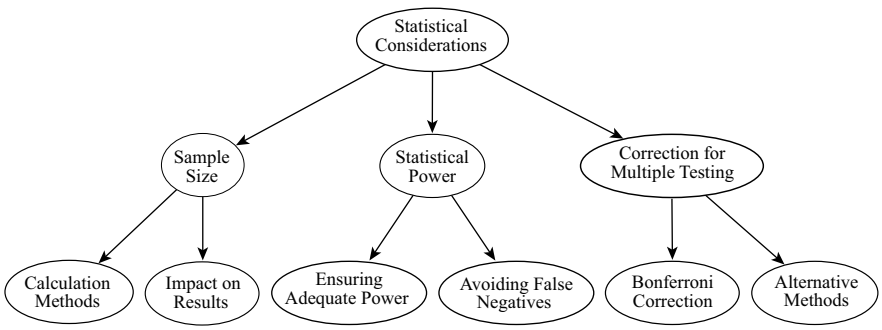

FIGURE 7.4

Flow chart of statistical considerations while preserving the power to detect true effects.

estimation multiple testing corrections will be to improve the validity of the study outcomes for the benefit of clinicians.

7.3 Cancer biomarker application in various cancers

Cancer biomarkers including specific characters, genes, or molecules provide precise patterns of disease development via early detection, prognosis, and diagnosis along with the choice of appropriate treatment options. Human epidermal growth factor receptor 2 (**HER2**) is well-identified biomarker that is overexpressing in around 20% of cases of breast cancer linked with inappropriate prognosis and aggression of disease. Patients with HER2-positive breast cancer are now experiencing significantly better results owing to the development of HER2-targeted treatments like trastuzumab (Herceptin) (Sarhadi & Armengol, 2022). By focusing on the HER2 receptor, trastuzumab suppresses the proliferation and survival of tumor cells, highlighting the significance of biomarker-driven therapies in improving clinical outcomes. In addition, mutations in the **BRCA1** (**BR**EAST **CA**NCER GENE **1**) and **BRCA2** (**BR**EAST **CA**NCER GENE **2**) genes are important indicators of breast cancer. These developed mutations enhanced the individualized risk management technique and simultaneously enhanced the ovarian and cancer risks. Prophylactic surgery has proven an applicable option to lower cancer risk in women with BRCA mutations. Even, BRCA mutation has therapeutic implications. PARP inhibitors like Olaparib have effectively treated malignancies with BRCA mutations, especially when tumor cells have compromised DNA repair systems (Das et al., 2024).

A new horizon in non-small cell lung cancer (NSCLC) as epidermal growth factor receptor (**EGFR**) mutations and anaplastic lymphoma kinase (**ALK**) rearrangements have revolutionized therapy approaches. EGFR mutation cases are reported, in approximately 50% of Asian populations and around 10%–15% of Western populations. Gefitinib and Erlotinib used in the targeted treatment, which inhibits the mutant receptor and leads to tumor growth restriction, are therapeutically highly effective for patients with EGFR mutations. Similarly, ALK rearrangement has proven to be a useful marker in about 5% of NSCLC cases (Zhou et al., 2024). ALK inhibitors like Crizotinib in ALK-positive NSCLC patients have shown exceptionally high efficacy in improving survival rate, without cancer progression, and overall, therapeutically effective outcomes. Molecular biomarkers have played an important role in enabling personalized cancer treatment, which already been demonstrated by the ongoing research and application of tailored medicine in the regimen (Rai et al., 2023).

For the selection procedure in the colorectal cancer treatment, essentially KRAS, NRAS, and BRAF gene's mutational statuses are prerequisites. They provide the prediction of mutation in these genes during Cetuximab and Panitumumab administration, due to resistance toward anti-EGFR treatments.

KRAS is an important predictive biomarker for colorectal cancer, with mutations found in about 40% of cases. Similarly, resistance to anti-EGFR therapy is also predicted by NRAS mutations, despite their lesser frequency. Reported studies show that BRAF mutations are found in almost 10% of colorectal tumor cases, especially V600E variant linked due to the poor prognosis. Identification of these mutations aids in the patient stratification and, even the selection of suitable treatment plans, such as targeted treatments that concentrate on the abnormal signaling pathways. Another essential biomarker for colorectal cancer is microsatellite instability (MSI) (Banin Hirata et al., 2014). High MSI status has been found in about 15% of colorectal tumors, which is found to be associated with the improvise prognosis and enhancing response to the immunotherapy. In an immune checkpoint inhibitor, pembrolizumab is one of the examples, which has been authorized for implication in the MSI-high colorectal tumors, and proven to show therapeutically significant outcomes. The use of biomarkers to direct immunotherapeutic methods is best illustrated by the integration of MSI testing into standard clinical practice (Ros et al., 2023).

Prostate-specific antigen (**PSA**) in oncology history is one of the oldest and most popular biomarkers. In benign illnesses, the elevated PSA levels observed in conditions like prostatitis or benign prostatic hyperplasia; it acts as an indicator of prostate cancer. Although the PSA has limitation in the specificity, still it is essential in prostate cancer diagnosis, screening, and monitoring purposes. Because of PSA accessibility and simplicity, it has been widely utilized in the clinical practices. In the therapy of metastatic castration-resistant prostate cancer, a prognostic biomarker for resistance has been identified, Androgen Receptor Splice Variant 7 (**AR-V7**) (Silsirivanit, 2019). Resistances to androgen receptor signaling inhibitors identifies with the presence of AR-V7, even Abiraterone and Enzalutamide, that directs the oncologists to acknowledge the alternative treatment of chemotherapy. Determining the AR-V7 status aids in avoiding ineffective medicines and customizing treatment plans, both of which improve patient outcomes (Abdulla & Leslie, 2024).

In ovarian cancer, Cancer Antigen 125 (**CA-125**) is an identified biomarker and particularly helpful in tracking disease recurrence. Identification of ovarian cancer relapse, and even CA-125 is useful marker in monitoring therapy response, because with the growth of epithelial ovarian tumor, CA-125 levels also increase. Nevertheless, CA-125 specificity and sensitivity are restricted, as in benign conditions and other cancers, its level rises. For the high accuracy and preciseness and enhancing the patient safety, for ovarian cancer additional biomarker identification research works are in progress. In supplement to the CA-125, Human Epididymis Protein 4 (**HE4**) has drawn interest. Ovarian cancer diagnosis has been improvised with the sensitivity and specificity, with the combination of HE4 with CA-125. There combination has led to significant impact by distinguishing between benign and malignant pelvic tumor, and even early detection and suitable treatment made easier by the Risk of Ovarian Malignancy Algorithm in cancer patients (Song et al., 2023).

Cancer Antigen 19-9 (**CA19-9**) in pancreatic cancer is the most widely utilized biomarker. Due to its nonspecific and high level observed during the cirrhosis and cholangitis, enhance level of CA19-9 could be the sign of the pancreatic cancer. Although it has limitations, it is considered well suitable and even utilized for optimizing the response in pancreatic cancer patient's treatment and during their disease treatment course. CA19-9 ability in identifying the clinical findings in the aggressive cancer treatment has emphasized its application in the biomarker-based monitoring (Kirti & Prabhakar, 2016) (Table 7.3).

Although the utilization of the cancer biomarker in the patients has led to tremendous improvement in the cancer treatment, even some challenges are still persisting. Therapeutic value of the biomarker is complicated due to the heterogeneity of tumor and dynamic nature of the biomarkers. Cancer diagnosis and treatment course could possibly affected by facllacious positives and negative responses results due to variation in biomarker expression within and between tumor interaction. Furthermore, biomarker testings are expensive, and in some region due to low resources, not ease to accessible and available to patients at large scale. Best technological development examples are NGS and liquid biopsies provide promising results in resolving these issues (Passaro et al., 2024). Comprehensive genomic profiling is made possible by NGS, which also provides a complete molecular landscape of tumors and the ability to discover many biomarkers at once. This methodology streamlines the process of identifying relevant mutations and provides guidance for customized treatment plans. A noninvasive technique for identifying and tracking cancer is provided by liquid biopsies, which examine circulating tumor DNA (ctDNA) and other indicators in blood. Through the real-time insights into tumor dynamics that liquid biopsies can offer, treatment response can be tracked and recurrence can be detected early. A major progress in the application of cancer biomarkers has been made with the invention and validation of liquid biopsy technologies, which have the potential to revolutionize cancer management. Apart from technological advancements, continuous investigation into new biomarkers keeps broadening the range of targets that can be taken action on. Incorporating novel biomarkers into clinical practice requires both their discovery and thorough validation studies. Translating biomarker research into real clinical benefits requires close collaboration between researchers, doctors, and regulatory agencies (Caputo et al., 2022).

The identification and analysis of cancer biomarkers has transformed many cancers' diagnosis, prognosis, and treatment, allowing for more individualized and efficient care. Biomarkers with substantial clinical usefulness, including as HER2, EGFR, BRCA1/2, KRAS, and MSI, have been shown to guide targeted therapy and enhance patient outcomes. Even though using biomarkers can be difficult, new developments in technology and continuing research could help to overcome these difficulties and improve the accuracy of cancer treatment. The advancement of cancer biomarkers and their incorporation into standard clinical practice will be essential to improve the prognosis of cancer patients across the globe (Nandi et al., 2020).

Table 7.3 Biomarkers identified in various cancers.

Type of cancer	Biomarker	Role	References
Breast cancer	BRCA1/ BRCA 2	Increased risk of genetic mutation; Insight into risk management and inhibitors of PARP	Momozawa et al. (2022), Ragupathi et al. (2023), BRCA2 BRCA2 DNA repair associated (2024)
	HER2	Indicates aggressive disease; target for trastuzumab (Herceptin)	Wang et al. (2022), Gajria and Chandarlapaty (2011)
Lung cancer	ALK	Reorganizing the target molecules in therapies like Crizotinib	Friboulet et al. (2014), Ou et al. (2012)
	EGFR	Target for the mutations for the treatment like Erlotinib & Gefitinib	Friboulet et al. (2014), Ou et al. (2012), Vyse and Huang (2019), Zhang (2016), Zou, Lee, and Yan (2018)
Colorectal cancer	BRAF	Recognizes the responses of specific treatments	Grassi et al. (2021)
	MSI	Indication of more appropriate forecasting & responding towards immunotherapy	Wilbur et al. (2024)
	KRAS	Envisages resistance towards anti-EGFR therapy	Misale et al. (2012), Giordano et al. (2019)
	NRAS	Identifying resistance towards anti-EGFR therapy	Prabhakar et al. (2020)
Prostate cancer	PSA	Responds towards disease screening and its monitoring	Alabi et al. (2023), Trantham et al. (2013)
	AR-V7	Recognizes the resistance towards the inhibition of androgen mediated transduction	Zhu et al. (2020), Zheng et al. (no date)
Ovarian cancer	CA-125	Monitoring of disease reoccurrence	Charkhchi et al. (2020), Bast (2010)
	HE4	Responses towards diagnostic specificity & sensitivity	Li et al. (2009)
Pancreatic cancer	CA19-9	Prediction of disease progression and monitoring of responses to the therapies	Lee et al.,at (2020), Ballehaninna and Chamberlain (2012)

7.4 Cancer biomarker regulatory pathway

EGFR (HER1), HER3, and HER4 are the other receptor tyrosine kinases of the ErbB family, which also contains HER2, commonly referred to as ERBB2. Numerous cellular functions, including survival, differentiation, and proliferation, are regulated by these receptors. The primary mode of action for HER2 is heterodimerization with other ErbB receptors; it does not have a recognized ligand. There are three primary domains on the HER2 protein: The *extracellular domain* is in charge of dimerizing other ErbB receptors, *transmembrane domain*that provides a cell membrane anchor for the receptor within cells, and *tyrosine kinase domain*thatupon activation; this domain is in charge of starting downstream signaling. Specific tyrosine residues in the intracellular domain of HER2 are autophosphorylated as a result of heterodimerization with other ErbB receptors, such as EGFR or HER3. Numerous downstream pathways are started by this autophosphorylation, which generates docking sites for different signaling proteins (Hsu & Hung, 2016; Wang, 2011).

Activation of HER2 triggers a number of vital signaling pathways that control several biological functions, including *PI3K/AKT Pathway*, the serine/threonine kinase AKT, which stimulates cell growth and survival, is phosphorylated and activated when the PI3K (phosphoinositide 3-kinase) pathway is activated. Moreover, AKT activity blocks apoptotic pathways, which prolongs the life of cancer cells. *The AS/RAF/MEK/ERK pathway*, sometimes referred to as the MAPK pathway, is triggered when phosphorylated HER2 binds to adaptor proteins such as GRB2 and SOS. The kinase cascade that involves RAF, MEK, and ERK is set off by the activation of RAS, a small GTPase, and results in the proliferation and differentiation of cells. *PLCγ Pathway*, diacylglycerol (DAG) and inositol triphosphate (IP3) are produced, with the activation of the phospholipase C gamma (PLCγ). DAG and IP3, respectively, act by the mediating the calcium release and activating protein kinase C (PKC). There are the numerous biological responses which are mediated through this route, such as survival and proliferation (He et al., 2021; Pan et al., 2024) (Fig. 7.5).

BRCA1 and BRCA2 are two of the most well-known genes, which are associated with the inherited ovarian and breast cancer. For homologous recombination, these genes generate the proteins, which is an important procedure for the repairing process of the damaged DNA. BRCA1 or BRCA2 mutations can compromise the repair pathway, one of the main reasons causing the cancer risk and creating genomic instability. Protein interactions are mediated by encoding a protein with important domains, including BRCT and RING domains for ubiquitin ligase activity. BRCA1 is found to be on chromosome 17q21. It plays essential part in various of biological roles, such as cell cycle regulation, transcription control, and repairing the damaged DNA. On chromosome 13q12.3, BRCA2 is located and encodes the protein that is responsible for attracting and functioning RAD51, essential step for the homologous recombination process. The protein is composed of a DNA-binding domain (DBD), along with the several BRC repeats. By the

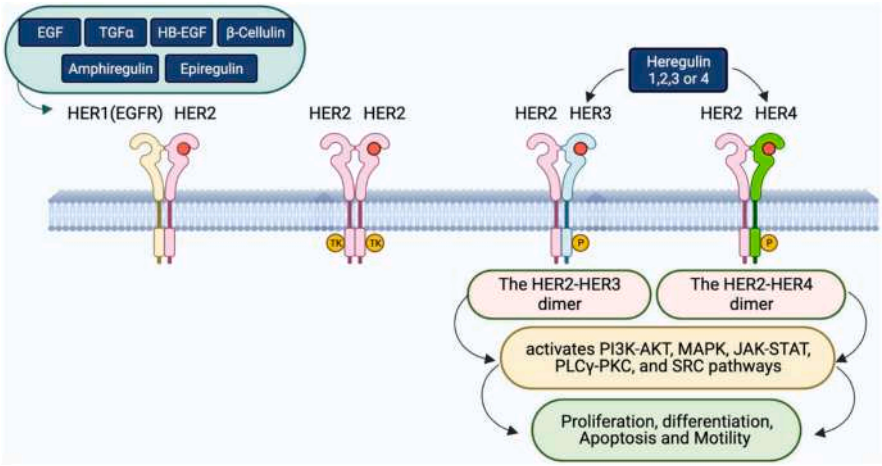

FIGURE 7.5

Dimerization and interactions among the HER receptor family members.

procedure of mending, double-strand DNA breaks down, and both genes are considered to be vital for maintaining the genomic integrity (DSBs) (Roy et al., 2012). In order to identify double-strand breaks (DSBs), BRCA1 detects DNA damage and helps in the formation of the complexes with proteins like ATM and MRN. It also enhances the activity of the resection with CtIP to produce, the single-stranded DNA with overhangs. By binding to ssDNA, and simultaneously attracting the RAD51 to the damage site, BRCA2 helps to find a homologous DNA sequence, that will help to guide the precise repair. Hormonal signals, transcription factors like p53, and epigenetic alterations like DNA methylation and histone modifications all tightly control the transcriptional level of expression of BRCA1 and BRCA2. Posttranslational alterations lead to impact the stability as well as the functioning of BRCA1 and BRCA2 proteins, which affect their efficacy in DNA repair processes. These modifications include the phosphorylation step by ATM and ATR kinases, ubiquitination by the RING domain, and acetylation and SUMOylation, respectively (Tan et al., 2023).

The extracellular domain (ECD), transmembrane domain, and intracellular tyrosine kinase domain make up the three major domains of EGFR, commonly referred to as ErbB1 or HER1. The four subdomains (I–IV) of the ECD are in charge of ligand binding. Subdomains II and IV aid in receptor dimerization, whereas subdomains I and III are involved in ligand binding. The receptor is anchored to the cell membrane by the transmembrane domain. Tyrosine residues on receptors and substrates are phosphorylated by the kinase activity of the intracellular tyrosine kinase domain. Several tyrosine residues in the C-terminal tail are phosphorylated following receptor activation and function as docking sites

for signaling molecules. Binding ligands like TGF-α and epidermal growth factor (EGF) starts the activation of EGFR. Conformational modifications brought upon by ligand interaction cause the autophosphorylation and dimerization of receptors. With other members of the ErbB family, such as HER2/ErbB2, EGFR can form homodimers or heterodimers (Rodriguez et al., 2023). The intrinsic kinase activity of the receptor cannot be activated without dimerization. Specific tyrosine residues on the C-terminal tail are phosphorylated by the kinase domains trans-auto during dimerization, forming docking sites for signaling proteins with Src homology 2 (SH2) or phosphotyrosine-binding domains. EGFR signaling is strictly controlled by degradation and endocytosis of the receptor. Activated EGFR is endocytosed via clathrin- or caveolae-mediated mechanisms, which either recycle the receptor back to the cell surface or drive it toward lysosomal destruction. By regulating lysosomal breakdown, ubiquitination avoids excessive or protracted signaling. Numerous important signaling pathways that control cellular processes like division, survival, and proliferation are triggered by activated EGFR (Zinkle & Mohammadi, 2018).

The engagement of the adaptor protein Grb2 and the guanine nucleotide exchange factor (GEF) SOS to phosphorylated EGFR initiates the RAS/RAF/MEK/ERK (MAPK) pathway, which is essential for cell division and proliferation. Subsequently, SOS encourages the trade of GDP for GTP on RAS, turning it on. The RAF kinases that are recruited and stimulated by the activated RAS phosphorylate and activate MEK, which phosphorylates and activates ERK. After activation, ERK translocates to the nucleus to control the expression of genes, so facilitating the division and multiplication of cells. Phosphoinositide 3-kinase (PI3K) is recruited and activated by phosphorylated EGFR, which initiates the PI3K/AKT pathway (Wang et al., 2024). PIP2 is changed into PIP3 by activated PI3K, which gives AKT docking sites. PDK1 and mTORC2 then phosphorylate and activate AKT. Activated AKT inhibits apoptotic pathways and controls a number of metabolic activities, hence promoting cell growth and survival. Phospholipase C gamma (PLCγ) is recruited and activated by phosphorylated EGFR through the PLCγ pathway. PIP2 is hydrolyzed by activated PLCγ to provide IP3 and DAG. DAG triggers the activation of PKC, whereas IP3 releases calcium from the endoplasmic reticulum. These occurrences regulate a number of biological functions, such as cell division and proliferation. Furthermore, Janus kinases (JAKs) may become activated in response to EGFR activation. Signal transducer and activator of transcription (STAT) proteins are phosphorylated by activated JAKs. After dimerizing and migrating to the nucleus, phosphorylated STATs control the production of genes vital to the growth and upkeep of cells. ALK is one receptor tyrosine kinase that is necessary for cell division and proliferation. Activated ALK initiates a variety of downstream signaling pathways that promote cell survival, proliferation, and metastasis (He et al., 2021).

The PI3K/AKT/mTOR pathway, the JAK/STAT route, and the RAS/RAF/MEK/ERK (MAPK) pathway are significant signaling pathways. By phosphorylating RAS, the MAPK pathway starts gene transcription and encourages cell development. This is followed by a cascade through RAF, MEK, and ERK. When

PI3K is activated, AKT is phosphorylated and activated via the PI3K/AKT/mTOR pathway, which promotes cell survival and proliferation. The JAK/STAT system controls the transcription of genes involved in immune response and cell proliferation by activating JAK kinases, which phosphorylates STAT proteins. The KRAS gene encodes KRAS4A and KRAS4B isoforms, which are members of the RAS family of small GTPases (Asati et al., 2016). These isoforms transfer signals from cell surface receptors to downstream effectors that are important in cell proliferation, differentiation, and survival. Through interactions between active KRAS and Ral-GEFs, the RAL pathway activates Ral proteins (RalA and RalB), regulating vesicular trafficking, actin dynamics, and gene expression to support cell growth and survival. Another member of the RAS family, NRAS, plays a critical role in signaling pathways that control the proliferation, survival, and differentiation of cells. GEFs such as SOS1 regulate NRAS activation by catalysing the conversion of GDP to GTP, which activates NRAS, and GTPase-activating proteins, which boost NRAS's intrinsic GTPase activity by hydrolyzing GTP to GDP and deactivating NRAS. Important signaling pathways like PI3K/AKT and RAF/MEK/ERK (MAPK), which are critical for cell growth, survival, and differentiation, are regulated by activated NRAS (Gentry et al., 2014; Thies et al., 2021).

Multiple functional domains of the protein kinase BRAF are tightly controlled to avoid excessive cell division and malignant transformation. Deactivation mechanisms and negative feedback loops are used to accomplish this regulation. A downstream element of the MAPK pathway called phosphorylated ERK can interact with scaffolding proteins and protein phosphatases to decrease RAF activity. Furthermore, BRAF's kinase domain can be dephosphorylated by protein phosphatases, which stops BRAF's catalytic activity. Preventing unchecked cell proliferation and preserving cellular homeostasis depend heavily on these regulatory mechanisms (Lake et al., 2016).

Androgen hormones, specifically testosterone and dihydrotestosterone, control the synthesis of PSAs. Prostate epithelial cells proliferate and differentiate more quickly in response to androgen stimulation, which increases PSA output. As a result, there is a correlation between blood PSA levels and both prostate size and androgen activity. Genetic mutations and androgen receptor signaling dysregulation can affect the regulatory pathways of PSA generation in prostate cancer. Elevated serum levels of PSA can result from prostate cancer cells producing PSA even when androgens are not present. Prostate tumors that are advanced or hormone-resistant frequently exhibit this characteristic (Harris et al., 2009).

The AR pre-mRNA must be spliced alternatively in order to produce AR-V7. The machinery of the spliceosome controls this process, which can lead to the skipping of exons that encode the AR LBD. Consequently, the transactivation domain and DBD of AR-V7 mRNA are retained but the LBD is absent, enabling ligand-independent binding and activation of target genes. Regulation of CA-125 production includes androgen deprivation therapy, genetic alterations, and hormonal environment in case of prostate cancer (Likos et al., 2022).

Several factors are known to affect the expression of CA-125, even if the precise mechanisms directing its creation are yet unknown. Hormonal modulation can affect CA-125 expression, especially changes in progesterone and estrogen levels. Furthermore, increased production of CA-125 by epithelial cells might result from inflammatory responses, raising blood levels. An additional element is tissue-specific expression, wherein the female reproductive tract's tissues mainly its epithelial tissues have higher concentrations of CA-125. The malignant transformation and cellular process dysregulation in ovarian cancer modify the regulatory pathways responsible for the generation of CA-125. CA-125 is frequently overexpressed by ovarian cancer cells, leading to elevated blood levels. Moreover, CA-125 is a valuable biomarker for disease surveillance and treatment response since it correlates with the tumor load and stage of ovarian cancer (Potenza et al., 2020).

WFDC2 encodes HE4, a secretory glycoprotein that is part of the WFDC domain-containing protein family. The regulation of HE4 production is influenced by several factors, including hormonal regulation, inflammatory response, and tissue-specific expression. In ovarian cancer, HE4 synthesis is often dysregulated due to malignant transformation and altered cellular processes. Overexpression of HE4 by ovarian cancer cells can lead to higher serum levels of the protein. HE4 has significant diagnostic utility, especially when combined with CA-125, enhancing diagnostic accuracy. This combination is particularly valuable in distinguishing ovarian cancer from benign diseases and monitoring disease progression (Hellstrom et al., 2010; Li et al., 2009).

The regulation of CA 19-9 production is intricate and poorly understood, impacted by both physiological and pathological conditions. Under physiological conditions, inflammation plays a role in the control of CA 19-9. In response to inflammation or tissue injury, which is triggered by inflammatory cytokines and mediators, epithelial cells may produce more CA 19-9. Moreover, the glycosylation processes that control the synthesis of CA 19-9 and its derivatives are influenced by the activity of glycosyltransferases, which include sialyltransferases and fucosyltransferases. The overexpression of CA 19-9 by cancer cells, which results in increased serum levels, is a characteristic of the regulation of the CA 19-9 biomarker in pancreatic cancer. Moreover, CA 19-9 levels are correlated with pancreatic cancer stage and tumor burden, which makes it a valuable biomarker for prognosis and disease monitoring (Timur et al., 2016; C-F Lee et al., 2023).

7.5 Conclusion

Cancer biomarkers have led to tremendous growth and development in the oncology field of medicine. Cancer biomarkers have proven their potential, which has led to a transformation in the cancer prognosis, diagnosis, and treatment and provided immense support toward personalized medicine. Although the cancer biomarker journey from discovery to the clinical stage and its application is

complex, it requires rigorous validation processes and adherence to stringent regulatory guidelines and pathways. Analytical and clinical validation guidelines guide the assessment of the biomarker's reliability, reproducibility, and clinical relevancy, along with adherence to regulatory guidelines and approval, and assure safety and therapeutic efficacy.

With the growth in the biomarkers field, an effective collaboration between the various subjective field researchers, clinicians, and regulatory bodies will be imperative and essential to overcome challenges such as assay standardization, data interpretation, and real-world validation. This will accelerate the science cutting-edge discoveries from the lab to bedside through clinical practice and enhance patient care and outcomes, by enabling precise and individualized treatment, cost-effectiveness, and improved survival rates.

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CHAPTER

Clinical trials and studies for biomarker evaluation

8

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8.1 Introduction

Biomarkers, defined by the National Cancer Institute, are biological molecules found in blood, body fluids, or tissues indicating normal or aberrant processes, conditions, or diseases like cancer. They differentiate between individuals with and without the disease, with changes attributed to factors like genetic alterations, transcriptional changes, and posttranslational adaptations. These biomarkers encompass proteins, nucleic acids, antibodies, and other categories, reflecting gene expression, proteomic, and metabolomic markers. Detectable in the circulatory system or body fluids, they enable convenient non-invasive assessments. Genetic biomarkers, inherited or somatic, can be identified through blood, sputum, or buccal cells or mutations in tumor tissue obtained via biopsy or specialized imaging.

In the contemporary landscape of precision oncology, where molecular profiling is increasingly accessible and affordable for individual patients, clinical trials are evolving to be guided by biomarkers with the primary aim of optimizing and personalizing cancer management. The central focus of these trials in cancer drug development is to enhance treatment efficacy, leading to meaningful clinical outcomes. The impact of clinical study results extends beyond individual patients, contributing to a deeper understanding of human biology and carrying long-lasting economic implications for healthcare and industry. However, the traditional progression from phase 1 to phase 3 is recognized as an established framework, yet the high failure rate in phase 3 underscores the limited accuracy of early-phase trials in forecasting benefits. This inefficiency results in participants undergoing futile treatments and wasted expenditures, despite the presence of potent experimental medications. The conventional drug development process, lasting an average of 12 years from pipeline to market, is deemed inefficient even

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with effective experimental drugs. Clinical trial designs are continually assessed and modified due to their critical role. Advances in genomics and cancer biology have identified a growing number of biological targets and associated therapeutics. Rapid evolution in oncology based clinical trial design is driven by our increasing capability to explore intricate data-intensive aspects of cancer biology at the individual patient level with shaping the application of targeted therapeutics. This chapter provides a comprehensive review on biomarker-driven oncology clinical trials, its design, and clinical utility as well as detail on prospects and outcomes.

8.2 Biomarker-driven oncology clinical trial

In recent years to optimize and personalize cancer treatment, significant progress has been achieved to develop targeted anticancer drugs. Traditionally, cancer patients were treated on the basis of tumor site and type. However, the need for specific oncology-targeted biomarkers is growing for accurate, precise, tissue-specific, and individualized cancer therapies for treating cancer patients due to recent developments in high-throughput next-generation genomic tumor profile sequencing and improvement in highly selective molecular targeted technologies. It is evidenced that about 90% of oncology drugs undergoing last-stage clinical trials failed to get market approval due to their inability to provide therapeutic benefits in clinical trials thus contributing not only to rising costs and slowing down the process of developing new anti-cancer drug but also responsible to deprive many cancer patients to potentially more effective treatment. Thus to mitigate the risk of this last-stage clinical trial failure, the US Food and Drug Administration (FDA) emphasizes the use of biomarkers by enriching the trial populations with specific molecular subtypes responding better to tested therapies. The growing use of precision medicine, which focuses on biomarkers or particular oncogenic mutations, has resulted in the development of innovative clinical trial designs that may assess the effectiveness of these medicines in a more comprehensive manner. Master protocols, like basket trials, umbrella trials, and platform trials, have the capability to assess histology-specific drug therapies that either target a shared oncogenic mutation in several tumor types or target several oncogenic mutation in single tumor type. Additionally, such protocols may also test for the presence of numerous biomarkers, rather than just one. Indeed, such protocols may expedite the drug assessment and appraise specific treatments targeted tumor categories that are currently not recommended. With rising prevalence of such intricate biomarker-based master protocols, it is imperative for advanced practitioners not only to comprehensive understanding of these innovative trial including their unique trial designs, recognizing their benefits and drawbacks, but also recognizing how their implementation might enhance drug development and optimize the therapeutic advantages of molecular precision medicine.

8.2.1 Basket trial

Basket trials are single or multiple arm open labeled phase-II clinical trials focus on screening of one treatment against a single molecular target irrespective of specific cancer types. The shared characteristic among participants in this trial is usually a predictive attribute that is determined by the pharmacology and mechanism of action of the intervention (Hobbs et al., 2022). A prerequisite for this basket trial is the presence of a specific biomarker targeted for multiple tumor histologies (Fig. 8.1A). Basket trials seek to inspect the idea that the existence of a molecular target or specific biomarker can act as an indicator of the

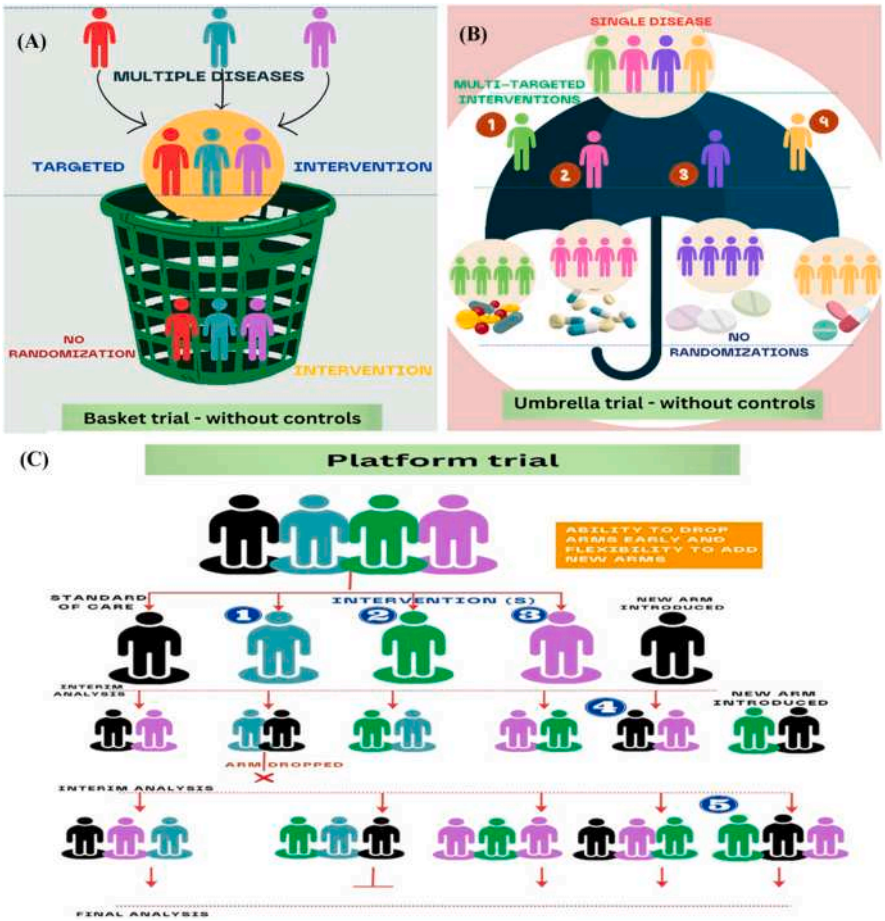

FIGURE 8.1

Biomarker driven oncology clinical trials (A) Basket Trial (B) Umbrella trial and (C) Plateform trial.

responsiveness and efficacy of a corresponding targeted therapeutic medicine, regardless of specific cancer type (Park et al., 2019).

An example of a basket trial is the VE-BASKET study, a phase II trial that aimed to evaluate the efficacy and safety of vemurafenib in nonmelanoma cancers, irrespective of their histology (Subbiah et al., 2019). Cohorts included NSCLC, cholangiocarcinoma, ECD/LCH, anaplastic thyroid cancer, breast cancer, ovarian cancer, multiple myeloma, colorectal cancer, and an "allothers" category with various malignancies. Remarkably, this trial marked the first FDA-approved basket trial (Diamond et al., 2018). Notably, ECD patients achieved a 54.5% overall response rate, with 2-year PFS and OS rates of 83% and 95%, leading to vemurafenib's FDA approval for ECD with a BRAF V600 mutation. While this trial secured approval for a specific disease, similar trials, like dabrafenib, expanded approvals, such as for unresectable or metastatic solid tumors with a BRAF V600E mutation after prior therapy progression (Moore & Guinigundo, 2023).

Basket trials, considered as compilations of enrichment experiments, share both pros and cons along with practical considerations. The challenge faced by basket trials involves finding a balance between practicalities and the ability to exchange hypotheses, particularly when dealing with histology-agnostic techniques, given the potential diversity among different illness types (Murciano-Goroff et al., 2024). Some biomarkers, due to their rarity, make it impractical to collect sufficient patient samples for each illness category. Aggregating patients based on the same biomarker may be questionable, assuming the disease subtype lacks prognostic significance. For those with BRAFV600 mutations, vemurafenib treatment efficacy varied significantly between melanoma and colorectal cancer as primary sites (Kelderman et al., 2015). To address this, merging certain illness categories with lower prevalence rates compared to others is an effective solution. An analysis was conducted separately for colorectal and non-colorectal cohorts due to the significantly higher occurrence in colorectal cancer.

Various adaptive designs have been devised to eliminate the necessity for distinct analyzes and effectively distribute response information across diverse illness types. One approach incorporates prearranged interim analyzes to guide subsequent actions (Hunsberger et al., 2009). If sufficient data suggests comparable and promising activity among cohorts with distinct histology, they are merged to enhance statistical inference with a reduced patient count. Conversely, histology-specific groups demonstrating highly positive responses are kept separate, while those with modest responses are discontinued (Kaizer et al., 2019). Another method employs statistical modeling and Bayesian inference, facilitating information exchange among cohorts with different histologies. This approach also allows for early study cessation for certain cohorts based on the posterior likelihood of histology-specific response rates (Liu et al., 2017). However, there is ongoing debate regarding the effectiveness of these designs, especially in phase II trials with small population sizes and limited cohorts or histology type (Cunanan, Gonen, et al., 2017).

Another crucial factor to consider in the design of discovery basket trials is the decision to employ randomization or not (Ratain & Sargent, 2009). The nonrandomized basket trial is highly advantageous due to its practicality and strong association with the typical single-arm phase II design commonly employed in the first stages of development. Nevertheless, in a nonrandomized basket design, the primary end point is typically limited to objective response, as it is widely regarded as the sole meaningful measure of efficacy in the absence of a comparison (Cunanan, Iasonos, et al., 2017; Rubinstein et al., 2005).

Additionally, there have been suggestions for multi-agent, nonrandomized basket trials, which essentially consist of a group of single-agent basket trials (Simon, 2018). These trials, facilitated by a centralized molecular screening platform, can be seen as umbrella trials. From a statistical perspective, there is a potential for multiplicity concerns in basket trials due to the simultaneous assessment of diverse histology-specific cohorts. While there may be a higher acceptance of the likelihood of false positive discoveries in practical terms, it is crucial to address concerns related to avoiding false-positive signals and bias as these trial designs progress (Kaizer et al., 2019).

8.2.2 Umbrealla Trials

Umbrella studies exemplify the utilization of biomarker-driven clinical trial designs in oncology. Umbrella trials, in contrast to basket trials, focus on a specific kind of cancer or histology but evaluate several biomarkers through screening and assessment (Fig. 8.1B) (Park et al., 2020). Such studies access its efficacy to evaluate effectiveness of various medicines, drugs and therapies. In umbrella trials, patients with diseases marked by multiple distinct biomarkers, such as non-small cell lung cancer (NSCLC) and breast cancer, are administered targeted drugs.

In the umbrella trial known as the plasma MATCH study, a phase II design explored diverse therapeutic strategies for metastatic breast cancer using liquid biopsy. The patients were categorized into five distinct cohorts and allocated to different treatments according on the presence (cohorts A, B, C, and D) or absence (cohort E) of biomarkers. Cohort A consisted of individuals who had a mutation in the estrogen receptor gene 1 (ESR1), and these patients were treated with fulvestrant (Faslodex) whereas Cohort B consisted of patients having a mutation in the human epidermal growth factor receptor 2 (HER2) gene, who were administered neratinib (Nerlynx). Cohorts C and D, exhibiting AKT mutations, received capivasertib in combination with fulvestrant and capivasertib monotherapy, respectively. Cohort E, comprising patients without identifiable mutations, received a combination of olaparib and ceralasertib (Turner et al., 2020).

In conducting umbrella trials, it is crucial to establish a predefined rule for matching biomarkers and candidate regimens. This proactive specification is essential for assessing the effectiveness of a rule-based policy in aligning biomarkers with drugs and determining the extent of success across various

genomic profiles (Khotskaya et al., 2017). Maintaining stability in the rule-based assignment policy throughout the trial is vital for ensuring the interpretability of evaluations and should encompass a broad spectrum of biomarker-drug combinations. Additional distinctive aspect of this trials is the potential eligibility of a patient for multiple biomarker cohort allocations, necessitating prospective determination of how to address this—whether through deterministic means (e.g., one biomarker taking precedence) or randomly (Simon & Polley, 2013).

8.2.3 Platform Trials

Platform trials, encompassing genetic profiling, basket trials, umbrella trials, and associated expansions, incorporate both nonrandomized and randomized enrichment strategies based on biomarkers in specified cohorts as shown in Fig. 8.1C (Woodcock & LaVange, 2017). These comprehensive trials comprise multiple enrichment sub-trials, each defined by molecular profiles, utilizing a centralized screening platform and shared data infrastructure. The approach allows flexibility in discontinuing unpromising investigations, advancing promising findings to phase II/III testing, and integrating new sub-trials as targets and agents evolve. Tailored for single-biomarker scenarios, these adaptive designs face distinct challenges, as illustrated by the evolutionary chronicle of two principal protocols, shedding light on hurdles encountered in executing these intricate trials (Meyer et al., 2020).

The NCI-MATCH is a program designed to pinpoint targeted cancer therapies based on tumor molecular characteristics (Khan et al., 2019). The trial, registered as NCT02465060 on ClinicalTrials.gov, is a basket trial integrating multiple targeted therapies. These trials fall under the master protocol category, employing nonrandomized or randomized enrichment designs based on biomarkers. The approach involves enrichment subtrials, defined by molecular profiles, with a centralized screening platform and shared infrastructure. The protocol offers flexibility, enabling termination of unpromising investigations and progression of favorable results to a phase II/III framework (Responders & Lung, n.d). Adaptive design methods for single-biomarker settings are used. Centralized trial management provides efficiency benefits. The study is ongoing, assessing 13 medications across 19 groups, with significant protocol revisions, including collapsing subtype cohorts to streamline the screening process (Flaherty et al., 2020).

In 2014, Lung-MAP was initially designed for advanced squamous NSCLC, featuring four targeted therapy subgroups and one cohort without specific treatment (Lam & Papadimitrakopoulou, 2018). Employing a seamless design integrating phase II and phase III trials, the primary endpoints were progression-free survival and overall survival (Ferrarotto et al., 2015). Subsequent to 2015, the landscape of advanced NSCLC therapy underwent substantial changes with FDA endorsements of multiple immunotherapies. These approvals significantly impacted treatment

quality and comparison groups in medical research, particularly for squamous and nonsquamous NSCLC (Lam & Papadimitrakopoulou, 2018). In 2018, a comprehensive overhaul occurred, involving minor modifications for biomarker-specific subtrials, broadening eligibility to encompass all advanced NSCLC histologies. A new screening process and additional biomarker-based subtrials were introduced to enhance the study's relevance and scope (Riess et al., 2021).

In summary, master protocols, despite their potential to improve efficiency and adaptability, face susceptibility to unforeseen challenges like low biomarker prevalence or unpredictable shifts in the treatment landscape throughout the trial.

8.2.4 Clinical Applications of Cancer Biomarkers: Examples

Cancer biomarkers have diverse therapeutic uses, aiming to achieve targeted therapeutics for optimal cancer prevention, screening, and treatment protocols. These applications include evaluating and analyzing potential risks, conducting initial screenings, detecting cancer at an early stage, providing accurate diagnoses, predicting patient outcomes, forecasting treatment responses, and tracking cancer progression. Moreover, a newly developed knowledge base named OncoMX has been established to enhance the investigation of cancer biomarkers by providing relevant evidence (Dingerdissen et al., 2020).

8.2.5 Biomarkers for exploring cancer risk assessment

Biomarkers indicating cancer susceptibility help identify individuals with a higher chance of developing cancer compared to the general population. Testing for cancer risk biomarkers involves DNA repair phenotypic assays and genotyping for germline variants. The interindividual variability in DNA repair is significantly linked to cancer susceptibility. Several technologies, such as the comet test, γH2AX foci generation, host cell reactivation assay, DNA repair signaling, and others, have been employed to assess DNA repair ability, damage, and response (Bahassi & Stambrook, 2014). Recent decades have witnessed the increased significance of genotyping tests due to advancements in high-throughput Next-Generation Sequencing (NGS) technology. In a large multicenter cohort study exploring the vulnerability of individuals to hereditary cancer syndromes and other diseases among a healthy population, 7.7% were found to harbor disease-predisposing variations linked to cancer syndromes (Schiabor Barrett et al., 2021).

After identifying and validating a biomarker that reliably indicates the risk of developing cancer, it is essential to integrate it into a comprehensive risk assessment model for the disease. This model should consider various factors, including environmental conditions and lifestyle choices, alongside the identified biomarker. Individuals identified as having an elevated susceptibility to cancer can choose to adapt their lifestyle and may benefit from increased monitoring, preventative surgery, or chemopreventive measures.

8.2.6 Biomarkers for screening and early cancer detection

Biomarkers play a crucial role in identifying cancer in asymptomatic individuals, promoting early detection for improved survival rates and reduced morbidity (Kumar et al., 2006). However, their use may lead to overdiagnosis, detecting cancers that may never cause symptoms (Dunn et al., 2022). Effective biomarker assays require high specificity, sensitivity, accuracy, low false positives, non-invasiveness, and cost-effectiveness to avoid unnecessary treatments and financial burdens (Sharma, 2009). Blood-based screening biomarkers, like those for liver, pancreatic, prostate, and ovarian cancers, vary in sensitivity and specificity (Louie et al., 2021). For instance, the PSA test for prostate cancer lacks specificity, prompting the exploration of novel tests, such as a filamin-A gene-based panel, showing promise in distinguishing benign and malignant conditions (Li et al., 2019). Ongoing initiatives aim to develop advanced liquid biopsy spectroscopy tests for multiple cancer types, surpassing current screening methods. However, concerns linger about their clinical feasibility, potential for overdiagnosis, unnecessary treatment, and precise tissue source determination (Cameron et al., 2023).

8.2.7 Biomarkers for diagnosis accurate cancer

Diagnostic biomarkers play a crucial role in definitively detecting cancer or specifying its type. These markers are invaluable for ensuring accurate diagnoses, facilitating effective therapy, and enhancing survival rates (Kaushal et al., 2022). While screening biomarkers can serve as diagnostic biomarkers, the former is typically applied to asymptomatic individuals, while the latter is specifically designed for those exhibiting symptoms. Despite their significance, diagnostic biomarkers alone are insufficient for a conclusive cancer diagnosis and should be supplemented with additional diagnostic methods like biopsies or imaging.

8.2.8 Patient Prognosis Biomarker

Upon tumor diagnosis, prognostic biomarkers play a crucial role in providing valuable insights into the disease trajectory, encompassing the recurrence likelihood, progression, and patient survival, irrespective of the treatment administered. Some biomarkers, including CEA for colorectal carcinoma, CA19-9 for pancreas cancer, and CA125 for ovarian cancer (Charkhchi et al., 2020), can also indicate the extent of cancer, aiding in staging, such as the tumor-node-metastasis (TNM) classification. Additionally, gene expression profiles, like MammaPrint and Prosigna, are utilized for breast cancer (Puppe et al., 2020). Genetic modifications allow precise categorization of acute leukemia patients, impacting outcomes (Inaba & Mullighan, 2020). The information derived from these biomarkers is instrumental for clinicians in deciding on aggressive or extended therapies. Notably, certain prognostic indicators are designed specifically to anticipate the benefits of chemotherapy in a therapeutic context.

8.2.9 Biomarkers as Predictive Tools for Personalized Cancer Therapy

Treatment decisions play a crucial role in managing cancer patients, and they are typically accompanied by uncertainty regarding the effectiveness, precision, side effects, and the risk of inappropriate overtreatment. However, notable breakthroughs are currently being made, and predictive biomarkers are rapidly playing a vital role in improving cancer treatment. This approach is founded on the idea that all notable breakthroughs are currently alterations in tumors or distinctive hereditary genetic variants (pharmacogenetics) that lead to a specific pattern of how cancer therapy medications affect the body. These biomarkers classify patients into responder and non-responder groups for various therapies, such as chemotherapy, hormone therapy, radiation, targeted methods, and immunotherapy. Moreover, certain biomarkers can predict individuals prone to significant medication toxicity, guiding dosage adjustments or alternative treatments for non-responders or those at heightened risk of adverse effects.

Biomarkers play a pivotal role in predicting tumor responses to conventional cancer therapies, such as chemotherapy and endocrine therapy. Limited biomarkers currently exist for this purpose (Batis et al., 2021). Germline variants on TPMT or TYMS genes are frequently employed as predictive biomarkers in cancer, while somatic cancer mutations also serve as valuable indicators for pharmacological treatment responses. Noteworthy progress has been made in creating multi-gene predictive biomarkers, exemplified by the Oncotype DX Breast Recurrence Score test, which examines 21 genes in breast cancer samples. This test aids clinicians in determining the most effective treatment for hormone receptor-positive and HER2-negative early-stage invasive breast cancer. Treatment options may include either endocrine therapy alone or a combination of chemotherapy and hormone therapy (Curtit et al., 2017). Additionally, the test offers insights into distant recurrence as a predictive biomarker, incorporated into the breast cancer staging system by the American Joint Committee on Cancer (Giuliano et al., 2017).

In radiotherapy field, the impact on cancer patients varies significantly, even among those with similar tumor types receiving comparable radiation treatments. Non-tumoral organs manifest differences in tumor response and the occurrence of early or delayed adverse reactions (Tamaddondoust et al., 2022). Various biomarkers, including molecular characteristics, specific mRNA molecules, proteins, DNA repair gene mutations, extracellular vesicles (EVs), and circulating tumor cells (CTCs), have been explored to assess tumor sensitivity to radiation therapy (Goodman et al., 2018). Studies on predicting radiation-induced toxicity in healthy tissues focus on DNA damage response, apoptosis, and germline genetic variants associated with various cancer types including prostrate and breast cancer (Fhoghlú & Barrett, 2019). Moreover, a correlation exists between blood protein biomarkers in breast cancer patients and the occurrence of cardiotoxicity following radiotherapy (Núñez, 2019).

In targeted cancer treatments, the initial crucial step involves pinpointing the specific genetic alteration driving tumor growth in a specific cancer type. Subsequently, a therapeutic approach is devised to address this modification, making targeted therapies effective only in malignancies possessing the precise genetic change targeted. To identify individuals who benefit from these treatments, biomarker assays, sometimes termed complementary diagnostics, are essential and are approved in conjunction with related medications (Scheerens et al., 2017). Companion diagnostic instruments, such as immunohistochemical tests or Next-Generation Sequencing, play a vital role in ensuring the safe and effective application of treatment. Specialized cancer biomarkers like the FDA-approved Foundation Focus CDx BRCA LOH (CDx BRCA loss of heterozygosity) assay aid in assessing risks and benefits, providing supplemental diagnostic information, as seen in the case of rucaparib for recurrent ovarian cancer. However, the use of such biomarkers is integral for personalized medicine but may not be required for general medication management (Ledermann et al., 2020).

Over the past decade, immunotherapy, particularly immune checkpoint inhibitors, has shown substantial effectiveness against certain cancers via boosting the body's immune response. Yet, the response varies among cancers, necessitating the development and optimization of biomarkers. PD-L1, microsatellite instabilities, and tumor mutational burden are FDA-approved biomarkers, with PD-L1 being a primary option, especially for NSCLC (Shen et al., 2019). Blocking the PD-L1 pathway with antibodies reactivates lymphocytes, enhancing antitumoral activity. Tumors with elevated PD-L1 expression show a positive response, but there is a need for improved precision and practicality in this biomarker (Wang et al., 2021).

8.2.10 Cancer biomarkers for the purpose of surveillance and monitoring treatment response

Periodically evaluated throughout the treatment process or post-completion, monitoring biomarkers play a crucial role in assessing various aspects, including current illness burden, disease deterioration, and therapy response. Among these, liquid biopsy biomarkers, notably ctDNA, are emerging as preferred options for minimal residual disease assessment and cancer surveillance (Heitzer et al., 2019). CtDNA, reflecting tumor presence over time, holds promise as a robust monitoring biomarker. In contrast, blood proteins like CEA and CA19-9, while commonly used, exhibit limitations compared to ctDNA. Key biomarkers for monitoring therapeutic response in lung cancer involve ctDNA driver mutations, alongside blood concentrations of Cyfra21-1 and potentially CA125 (Duffy, 2023). Despite their significance, the practical implementation of monitoring biomarkers faces challenges due to methodological and biological constraints (Athanasios Armakolas et al., 2023).

8.3 Issues and Prospects

Oncology is characterized by its multidisciplinary nature. Nevertheless, the assessment of multimodality regimens that go beyond basic combinations is still challenging due to several obstacles, such as the need for suitable controls and blinding. The utilization of biomarkers exhibits significant promise, although presently suffers from a lack of uniformity across the proliferating technologies. Radiomics is a developing category of biomarkers that involves the extraction and analysis of quantitative data from imaging, showing great potential. There is still a need for clinical trials that use atypical biomarkers and assess various treatment methods in diverse combinations and sequences, specifically in the context of targeted therapy. Furthermore, it is crucial to give equal consideration for scrutiny various clinical trial designs and their corresponding approvals by regulatory authorities such as the FDA. Additionally, advancement of clinical trial designs based on a more refined concepts of cancer biology.

Large health care centers are ideally equipped to provide the necessary robust and specialized infrastructure for current and future clinical studies. Nevertheless, providing medical care to patients in their local communities offers both convenience and the opportunity to establish strong relationships based on geographical proximity. It is worth noting that the bulk of cancer care is administered within these areas. This gives a chance to connect and utilize the advantages of these two platforms. Efforts involve the use of centralized analysis and remote Medical therapy Boards (MTBs) to provide therapy in the community. This can lead to a cooperative partnership across different healthcare settings.

Ultimately, the clinical trial is an essential component in the field of oncology. The conventional phase I to III trial model has been established the majority of our existing standard-of-care therapies. Nevertheless, as new therapeutic treatments and biomarkers continue to advance at a fast pace, a distinct approach is emerging and being implemented.

Our present understanding of cancer biology and relevant targeted therapy is integrated into primary protocols. Thus, clinical trial designs will also need to progress as comprehension of cancer biology progresses. Integrating developing concepts like complete molecular profiling, machine learning, and real-world data into trial designs, along with existing multimodality treatments, will be crucial for providing optimal care to cancer patients. Finally, the design of a clinical trial is but one component of the trial. Equal attention should be given to guaranteeing the soundness of clinical trial execution, accurate analysis of the findings, and implementation in real-world medical settings.

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CHAPTER

Early detection and diagnostic biomarkers

9

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9.1 Introduction

It has been known for a long time that early diagnosis of cancer is key to better care and results for patients. Finding cancer early makes it more likely that it will be localized and respond well to treatment, which is good for the long run. For example, more than 90% of people with localized breast cancer will still be alive after 5 years, but less than 30% of people with breast cancer will still be alive after 5 years (Etzioni et al., 2003). Early detection lowers the need for harsh treatments, which not only raises the chance of surviving but also lowers the risk of patient illness and healthcare costs (Jemal et al., 2011). Effective screening programs that target early detection have led to significant decreases in the death rates of some malignancies, including colorectal, cervical, and breast cancers. Pap smears, colonoscopies, and mammograms have all proved crucial in detecting these malignancies in their early stages, frequently before the appearance of symptoms. For example, people who get routine screening had a 68% lower death rate when colorectal cancer is discovered early thanks to colonoscopies (Ries et al., 2003). Innovations in technology, such as low-dose computed tomography (LDCT) for lung cancer screening, have also proven to be effective. Research indicates that the mortality rate from lung cancer can be reduced by up to 20% in individuals who are at high risk, particularly heavy smokers, through the use of LDCT screening ("National Lung Screening Trial Research Team et al. Reduced lung-cancer mortality with low-dose computed tomographic screening," 2011). However, these methods are not particularly beneficial due to their invasive nature, high cost, and occasional inadequacy. As a result, there is increased interest in the creation of highly specific but noninvasive diagnostic biomarkers for early cancer diagnosis. The identification of biological alterations that take place in the initial phases of cancer development is critical to the effectiveness of early detection programs. This concept functions as the cornerstone of biomarker research, which endeavors to identify specific biological markers that are associated with the presence of cancer, often before clinical symptoms appear (Hanahan & Weinberg, 2011). In the subsequent section, the role of diagnostic biomarkers in this critical process are investigated.

The Potential of Cancer Biomarkers. DOI: https://doi.org/10.1016/B978-0-443-29279-8.00009-0

As the name suggests, a diagnostic biomarker is a biological sign that shows that cancer exists. They are used for clinical tests because they can be found in blood, urine, and biopsies, among other biological fluids and tissues. Biomarkers are very important for finding cancer early because they send molecular signs that can be picked up before the disease gets fully developed (Sawyers, 2008). Biomarkers play a big role in the technology that finds cancer without or with little harm to the body. They show a lot of promise for making imaging or tissue biopsies better as screening tools. PSA, which stands for prostate-specific antigen, is a well-known test that can be used to find prostate cancer early. Even though there is discussion about overdiagnosis and overtreatment, PSA testing is still an important way to find prostate cancer early (Loeb et al., 2014). Cancer antigen 125 (CA-125) is another often-utilized clinical marker in ovarian cancer instances. However, concerns have been voiced regarding the sensitivity and specificity of these markers, which is why scientists are always searching for markers with higher accuracy (Jacobs et al., 2016). Thanks to advancements in proteomics, metabolomics, and genomics, there are now more biomarkers available for the detection of cancer. Common examples of genetic biomarkers are alterations in gene expression or mutations connected to various cancer types. Finding BRCA1/BRCA2 mutations using high-throughput sequencing technology has very much changed how cancers like breast cancer are found since these genes are closely linked to a higher risk (Bettegowda et al., 2014). Circulating tumor DNA (ctDNA), which is found in the blood, is another interesting biomarker that is being used in liquid biopsy methods to find cancer without surgery (Sacher et al., 2016). Personalized oncology is a bigger area where diagnostic biomarkers are important for more than just finding cancer early. By finding molecular signatures, biomarkers help doctors make cancer medicines that are specific to each patient's body. Today, biomarker research is very important for cancer treatment because this personalized method makes treatment more effective and less harmful (Verma, 2012).

9.2 Types of cancer biomarkers

Cancer biomarkers are broken down into four groups to help with detection, monitoring, and treatment. These groups are diagnostic, prognostic, predictive, and pharmacodynamic. Each group is important for diagnosing, treating, and managing cancer in its own way (Zhou et al., 2024). The main job of diagnostic biomarkers is to find cancer, normally when it is still very early on. They do this by pointing out abnormal molecular or biological processes that may show the start of cancer before symptoms show up. For example, checking blood levels of the PSA has been useful in screening for prostate cancer (Loeb et al., 2014). Another illustration is the ovarian cancer diagnostic tool, CA-125, which is more successful when used in conjunction with other diagnostic instruments (Jacobs et al., 2016). Prognostic biomarkers provide information about how a malignancy will probably progress or

turn out in the absence of therapy. Because they show how the tumor is behaving biologically, they help predict how the illness will progress and what the patient's general outlook is. Human epidermal growth factor receptor 2 (HER2) overexpression is a well-known sign of a more lethal type of breast cancer (Slamon et al., 1987). These are very important because they let doctors put patients into groups based on their risk and pick the right level of treatment rigor. As long as doctors have predictive biomarkers, they can guess how a patient will react to a medication. They are necessary for personalized oncology, in which treatments are tailored to each patient's unique biomarker makeup. The use of EGFR (epidermal growth factor receptor) mutations in lung cancer, which indicate tyrosine kinase inhibitor susceptibility, is one such example (Mok et al., 2009). Similarly, in colorectal cancer, KRAS mutations predict not responding to anti-EGFR treatments such as cetuximab (Douillard et al., 2013). Thus predictive biomarkers are essential for maximizing therapeutic approaches, avoiding pointless procedures, and reducing unfavorable side effects. Biomarkers for pharmacodynamics show how the body reacts to a treatment. These signs help doctors keep track of how well therapy is working in real time, so they can change treatments as needed. One way to see how well-targeted treatments work on metastatic tumors is to look at the amounts of ctDNA. A drop in ctDNA levels during treatment is a sign of a good therapeutic reaction, so pharmacodynamic indicators are very important for keeping an eye on how well the treatment is working (Bettegowda et al., 2014). All of these types of biomarkers are important for managing cancer at different times, from finding it early to keeping track of it and tailoring treatment to each person.

9.2.1 Sampling techniques for cancer biomarkers

The versatility of biomarkers makes them applicable to a wide range of biological materials. This opens up a world of possibilities for their application; non-invasive or minimally invasive sampling techniques are within reach, and Biomedical samples such as blood, urine, tissue biopsies, saliva, and stool are frequently utilized for the purpose of identifying cancer biomarkers. One of the most popular materials for biomarker testing is blood, especially when it comes to liquid biopsies. Through the analysis of circulating biomarkers, such as exosomes, ctDNA, and circulating tumor cells (CTCs), liquid biopsy is able to detect the presence of cancer without requiring tissue samples (Diaz & Bardelli, 2014). This noninvasive method may be used to track the development of the disease, identify early signs of malignancy, and assess how well a treatment is working. Biomarkers can also be found in urine, particularly in the case of urological cancers such as prostate and bladder cancer. Noninvasive urine-based biomarker testing can reveal information on molecular alterations in the genitourinary tract. Prostate cancer gene 3 (PCA3) and other urine biomarkers have demonstrated potential for early prostate cancer identification (Groskopf et al., 2006). The most reliable method for identifying biomarkers and diagnosing cancer is still tissue biopsies. Through a conventional

tissue biopsy, specific markers, such as overexpressed proteins and genetic anomalies, can be found by closely examining tumor cells. Tissue samples, however, are invasive and may not necessarily provide a comprehensive view of the genomic landscape of the tumor due to tumor heterogeneity (Gerlinger et al., 2012). Recent developments in minimally invasive methods, like core-needle biopsy and fine-needle aspiration, have lessened the trauma associated with tissue samples for patients. Stool samples are collected for colorectal cancer screening, and saliva is analyzed for oral malignancies. Blood or stool-based assays that look for DNA alterations are frequently utilized for colorectal cancer early detection (Imperiale et al., 2014). The more intrusive screening techniques, such as colonoscopy, can be substituted with these noninvasive procedures. Cancer biomarkers can be applied in a variety of ways to reduce patient suffering and risk while detecting cancer early, diagnosing it, and monitoring its course of treatment. This is accomplished by employing a variety of biological products and sample collection techniques.

9.3 Technologies for biomarker discovery

Thanks to the rapid expansion of proteomics, metabolomics, and genomics technologies, the hunt for cancer biomarkers has made a great headway. Since every one of these disciplines offers a unique perspective on the molecular alterations occurring in cancer, the hunt for biomarkers to support diagnosis, prognosis, and treatment choice has become simpler (Tainsky, 2009). Examining the entire genome, including mutations, changes in gene expression, and epigenetic alterations associated with cancer's progression, is one of the primary aims of genomics research (Mardis, 2013). Recent advances in high-throughput technology, such as next-generation sequencing (NGS), have revolutionized genetics by rapidly sequencing massive volumes of DNA extracted from cancer cells. These technologies make it possible to quickly sequence big amounts of DNA from cancer cells. For example, hereditary ovarian and breast malignancies have been associated with specific mutations in the BRCA1 and BRCA2 genes. The identification of these mutations has been largely facilitated by NGS (Miki et al., 1994). As indicators of cancer, genomic technology can identify mutations, copy number alterations, gene fusions, and microsatellite instability. Proteomics is the broad study of proteins, which are the fundamental building blocks of all cells. Proteomics tools such as mass spectrometry enable studies of protein expression levels, post-translational modifications, and protein-protein interactions in cancer cells (Aebersold & Mann, 2003). Protein composition changes in cells could be an indication of cancer or a warning that the illness is getting worse. Proteomics is widely used in biomarker identification; two of the most well-known examples are high levels of particular proteins, such as CA-125 in ovarian cancer and PSA in prostate cancer (Bast et al., 2002). Proteomic research has helped to identify several

new cancer biomarkers, therefore facilitating the development of customized treatments. Small molecules, also referred to as metabolites, generated by biological metabolic activity form the focus of metabolomics research. Reflecting the changing metabolic activity inside cancer cells, metabolites reveal the metabolic reprogramming under carcinogenesis. By means of metabolic profiles of cancer cells, one can identify particular metabolic modifications functioning as biomarkers for early diagnosis and prognosis (Spratlin et al., 2009). Metabolomics has been used to detect variations in glycolysis and lipid metabolism in a variety of cancer types (Ward & Thompson, 2012), therefore guiding the identification of new targets for therapeutic intervention and the biomarker search. Taken together, all these instruments form the basis of modern biomarker research since they offer different but complementary viewpoints on the molecular changes defining cancer. Fig. 9.1 illustrates the comprehensive biomarker discovery process for early cancer

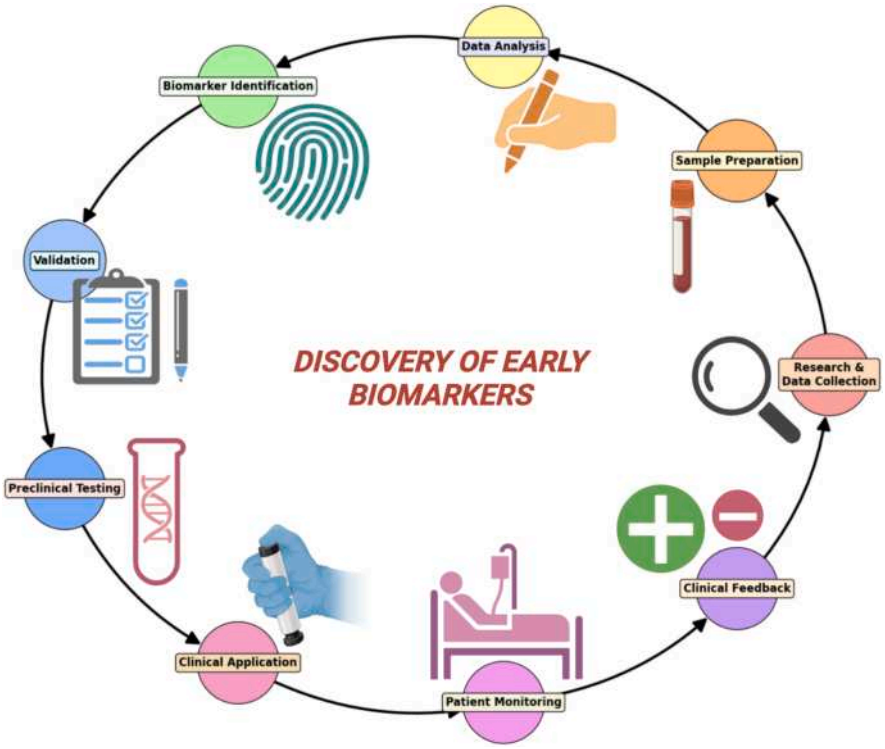

Figure 9.1 Detailed biomarker discovery process for early cancer detection

This figure illustrates the comprehensive biomarker discovery workflow, from initial research and data collection to clinical application and feedback. Each stage highlights the iterative nature of biomarker validation, emphasizing the integration of genomics, proteomics, and metabolomics technologies for enhanced cancer detection and personalized oncology approaches.

detection, depicting the interconnected stages from initial research and data collection through clinical feedback, highlighting the iterative nature of the workflow.

The development of liquid biopsy is a significant advancement in the noninvasive monitoring and diagnosis of cancer. Liquid biopsies, as opposed to invasive and frequently surgical traditional tissue biopsies, analyze CTCs, ctDNA, and other components generated from cancer that are present in bodily fluids such as blood (Diaz & Bardelli, 2014). This slightly invasive method gives information about how tumors change over time and lets doctors keep an eye on cancer in real time without having to take multiple tissue samples. Liquid biopsy has been shown to be very helpful for checking on the growth of metastasized tumors and seeing how well a treatment is working (Alix-Panabières & Pantel, 2016). One of the key advantages of liquid biopsy is its ability to detect ctDNA, which carries genetic changes akin to those in the original tumors. This enables the early diagnosis of cancer recurrence and the identification of genetic alterations that may result in resistance to targeted medications (Bettegowda et al., 2014). Liquid biopsy adds to precision oncology by finding biomarkers in patients whose tumors might not be easy to get to for tissue samples. Imaging methods like positron emission tomography (PET), magnetic resonance imaging, and computed tomography (CT) have been very important for finding cancer and keeping an eye on it for a long time. However, progress in molecular imaging has made imaging even more useful in the search for biomarkers. Radiolabeled chemicals or contrast agents that bind to certain cancer biomarkers are used in molecular imaging methods to see tumors at the molecular level (Gambhir, 2002). For example, increased glucose absorption in cancer cells has been commonly detected by PET imaging with fluorodeoxyglucose, which is indicative of metabolic reprogramming observed in many malignancies. Additional molecular imaging methods, such as those that focus on PSMA (prostate-specific membrane antigen) in prostate cancer and HER2 expression in breast cancer, offer important insights into the biology of tumors and aid in the development of individualized treatment plans (Lütje et al., 2015). As liquid biopsy and molecular imaging become more common in clinical practice, they are changing how cancer is found, tracked, and treated. These ways of finding biomarkers are noninvasive and very specific. Table 9.1 lists the main tools used to find biomarkers, the types of biomarkers that are found, and how they can be used to diagnose and treat cancer.

9.4 Clinical applications of diagnostic biomarkers

Diagnostic biomarkers have fundamentally revolutionized the way cancer is identified since they allow early-stage tumor diagnosis and enhance patient outcomes. Diagnostic biomarkers have shown great value in clinical settings according to several case studies including several kinds of cancer (Pulumati et al., 2023). One well-known example is

Table 9.1 Technologies for biomarker discovery in cancer detection.

Technology	Description	Key Biomarkers discovered	Application in cancer
Next-generation sequencing (NGS)	High-throughput sequencing to identify genetic mutations	BRCA1/BRCA2 mutations	Hereditary breast and ovarian cancers
Proteomics (mass spectrometry)	Large-scale study of proteins and their functions	PSA, CA-125	Prostate and ovarian cancer detection
Metabolomics	Analysis of small molecules/metabolites produced in cells	Altered lipid metabolism markers	Early detection in various cancer types
Liquid biopsy	Non-invasive sampling of circulating tumor DNA (ctDNA)	EGFR mutations, KRAS mutations	Early lung and colorectal cancer detection

the early prostate cancer diagnosis achieved by means of PSA. PSA, a protein generated by benign as well as malignant prostate gland cells, can be measured using blood. Rising PSA levels could indicate prostate cancer and call for further diagnosis-oriented testing. The PSA test has been widely used as a prostate cancer screening tool, despite considerable debate regarding its specificity. PSA screening has been demonstrated in studies to lower the death rate from prostate cancer, yet there is still a danger of overdiagnosis (Thompson & Ankerst, 2007). Overdiagnosis is the term used to describe the discovery of malignancies that would not have caused damage to a patient and may have led to unnecessary medical intervention. Another example is the diagnostic instrument for ovarian cancer, CA-125 (cancer antigen 125). CA-125 levels are typically increased in women with ovarian cancer, especially as the disease progresses. CA-125 has been shown to be useful when paired with other diagnostic modalities, such as transvaginal ultrasonography, despite the fact that its higher frequency in other illnesses (such as endometriosis) may make it insufficiently specific for early detection. This combination has enhanced the ability to detect ovarian cancer at an earlier stage, thereby increasing the probability of successful therapy (Jacobs et al., 2016). The analysis of ctDNA in lung cancer has become a viable diagnostic for early detection. Liquid biopsy techniques that analyze ctDNA can identify genetic alterations associated with lung cancer, enabling a more personalized treatment approach and an earlier diagnosis (Bettegowda et al., 2014). For patients with non-small cell lung cancer, the identification of EGFR mutations in ctDNA is being utilized to guide targeted therapy, providing a noninvasive substitute for tissue biopsy (Sacher et al., 2016). Another excellent example is the case of breast cancer. HER2 overexpression in breast cancer has been linked to the development of targeted therapies such as trastuzumab (Herceptin), which has revolutionized the treatment of

HER2-positive breast cancer, in addition to its use as a diagnostic and prognostic biomarker (Slamon et al., 1987). The fact that HER2 testing is now commonly done on breast cancer patients to see if they are eligible for targeted treatment shows how useful biomarkers are in personalized oncology as a therapeutic tool.

9.4.1 Advancements in biomarker-driven cancer screening programs

Biomarker-driven screening programs try to find cancer early in people who don't have any symptoms. The majority of these projects use biomarkers to identify high-risk individuals or detect cancer early. Typically, they are designed to target particular cancer types. Biomarker-based cancer screening systems may reduce false positives and negatives while simultaneously improving cancer detection rates. With the use of biomarkers like ctDNA, LDCT screening for lung cancer has become increasingly successful. While LDCT is a useful tool for identifying suspicious tumors, it is not necessarily specific to malignancy. The ability to distinguish between benign and malignant nodules using liquid biopsy techniques can significantly increase the precision of lung cancer screening ("National Lung Screening Trial Research Team et al. Reduced lung-cancer mortality with low-dose computed tomographic screening," 2011). If ctDNA analysis and LDCT are used together, the review process might go faster and doctors might not have to do as many tests on their patients because they are not needed. Making early screening tests for multiple cancers (MCED) is another important achievement. These tests employ biomarkers in the blood to detect multiple types of cancer at once. The Galleri test, which looks at methylation patterns in ctDNA, is a novel technique to detect cancer early on. It simply takes one blood sample to detect more than 50 distinct types of cancer (Klein et al., 2021). MCED tests are clinically useful because they bridge a major gap in current screening tactics by being able to detect tumors like pancreatic and ovarian cancers, for which there are no standard screening methods. Colonoscopy and fecal occult blood tests have been the foundation of colorectal cancer screening programs for a long time.

9.4.2 Expanding biomarker use in other cancer screening methods

However, stool DNA testing has made it easier to detect precancerous lesions and colon cancer in their early stages. These tests seek for alterations in several genes, including the APC and KRAS. If someone does not want a colonoscopy, these non-invasive diagnostics can be utilized instead (Imperiale et al., 2014). Mammography has also been used with biomarker studies that involve circulating cancer cells (CTCs) and ctDNA to improve the sensitivity and specificity of screening. The greatest advantage of biomarker-driven screening is shown in women with dense breast tissue, whose traditional mammography may miss microscopic tumors (Bardia & Haber, 2014). Overall, biomarker-driven screening programs have the potential to revolutionize the field of cancer diagnosis by offering more accurate,

Table 9.2 Diagnostic biomarkers for early detection of cancer.

Biomarker	Cancer type	Sensitivity	Specificity	Clinical application
PSA (prostate-specific antigen)	Prostate cancer	High	Low	Used in routine screening but can lead to overdiagnosis
CA-125	Ovarian cancer	Moderate	Low	Effective when combined with other modalities like ultrasound
EGFR mutations	Non-small cell lung cancer (NSCLC)	High	High	Guides the use of targeted therapies in NSCLC
BRCA1/BRCA2 mutations	Breast and ovarian cancer	High	High	Identifies genetic predisposition, informs preventative measures

tailored, and noninvasive methods to identify malignancies at the earliest, most treatable stages. The success and popularity of these screening programs are expected to increase as biomarker research progresses. The clinical applicability, sensitivity, and specificity of the diagnostic biomarkers used in the early detection of various cancers are shown in Table 9.2

9.5 Challenges and future directions

Despite the great potential that cancer biomarkers hold for early diagnosis, treatment guidance, and detection, several barriers stand in the way of their widespread clinical application. The primary concerns are achieving enough sensitivity and specificity and addressing the problem of biological heterogeneity in tumors (Chehelgerdi et al., 2023). Specificity is a biomarker's ability to correctly identify people who don't have the disease, while sensitivity is a biomarker's ability to reliably identify people who do have the disease. To make a useful diagnostic tool, we need to find a balance between these two points of view. While a highly specific test minimizes false positives (incorrectly diagnosing the disease), a highly sensitive test lowers the probability of false negatives (missing the disease) (Parikh et al., 2008). Nevertheless, a lot of cancer biomarkers have trouble achieving high specificity as well as sensitivity. For instance, PSA testing for prostate cancer has a low specificity despite its high sensitivity, which causes overdiagnosis and overtreatment of indolent tumors that may not have needed medical attention (Cary & Cooperberg, 2013). Another example is the biomarker CA-125, which is commonly high in non-malignant diseases such as endometriosis in ovarian cancer. False hits arising from this would reduce the test's accuracy (Jacobs et al., 2016). Scientists are creating more sophisticated biomarker panels with many biomarkers to increase sensitivity and accuracy as certain biomarkers are not very specific.

Different areas of the same cancer might have distinct metabolic, proteomic, or genetic pattern (Gerlinger et al., 2012). This is because cancer is not a single disease. Because of this range, it is harder to make reliable biomarkers because they might not fully show all the genetic changes that happen in a tumor. Therefore, even within the same cancer type, a biomarker that is effective for one subset of patients may not be useful to another population. Advanced or metastatic malignancies especially show this problem since the molecular markers of various metastases may differ from those of the original tumor (Sottoriva et al., 2010). To overcome these obstacles, multiparameter biomarker tests that can consider tumor heterogeneity and offer a more complete picture of the disease are required. Moreover, the issue of heterogeneity could potentially be addressed through the long-term tracking of biomarkers through techniques like liquid biopsy, which offer instantaneous insights into the development of tumors (Diaz & Bardelli, 2014). The development of personalized oncology, in which a patient's tumor's molecular characteristics inform treatment choices, is critical to the future of clinical application and research into cancer biomarkers. Since biomarkers provide the molecular information needed to choose a medicine, predict a treatment's outcome, and monitor the disease's progression, this approach mostly depends on them.

9.5.1 Overcoming sensitivity, specificity, and tumor heterogeneity challenges in biomarker research

NGS technology and liquid biopsy are being used to create unique genetic profiles for each cancer patient. This is an interesting area of study. These ways look at ctDNA, CTCs, or other signs in the blood to find specific genetic problems, gene fusions, and other changes that are making cancer worse (Bettegowda et al., 2014). Physicians can choose individuals most likely to benefit from focused treatment who have mutations in EGFR, ALK, or BRAF (Mok et al., 2009). The development of artificial intelligence and machine learning will also change our quest for cancer treatments and diagnosis approaches. Algorithms that employ artificial intelligence have the capacity to analyze vast quantities of biological data and identify patterns and connections that would be overlooked by conventional methods. These technologies contribute to the improved accuracy and personalization of cancer diagnoses by predicting the biomarker combinations that are most indicative of the disease (Esteva et al., 2017). AI is also being used to develop prediction models for treatment outcomes based on biomarker profiles, which will enhance the delivery of precision medicine. Another interesting advance in personalized oncology is the concept of multicancer early detection (MCED) (Liao et al., 2023). MCED assays combine markers, including methylation patterns in ctDNA, to identify many cancer types from a single blood test. By detecting tumors like pancreatic and ovarian cancers, which presently lack efficient early detection techniques, these tests have the potential to completely transform cancer screening (Klein et al., 2021). It is anticipated that a more comprehensive understanding of cancer biology

will be possible once genomes, proteomics, metabolomics, and immunomics are incorporated into standard therapeutic practice. By integrating data from these various molecular domains, researchers can develop multi-omics techniques that offer a more comprehensive understanding of cancer and identify biomarkers that represent the complex interplay of genetic, protein, and metabolic alterations in malignancies (Hasin et al., 2017). Despite the fact that there are still challenges with sensitivity, specificity, and tumor heterogeneity, ongoing technological advancements and biomarker research are establishing the foundation for a future in which personalized oncology will be the standard for cancer treatment. Biomarkers will continue to be indispensable in this transition, as they will guide the selection of personalized treatment plans that improve patient outcomes, in addition to facilitating early diagnosis and detection.

9.6 Liquid biopsy: a noninvasive frontier in early detection

Rising as a breakthrough noninvasive method for early cancer detection is a liquid biopsy. Analyzing tumor-derived materials including exosomes, found in blood and other body fluids, ctDNA, and CTCs is part of it. Liquid biopsy provides a more easily available, real-time approach for tracking tumor dynamics, thereby capturing the genetic evolution of cancer with minimum patient risk than conventional tissue biopsy, which is invasive and often limited by sample availability (Bettegowda et al., 2014). Liquid biopsy's fundamental idea is to find cancer biomarkers floating about the circulation. Growing tumors lose cells and genetic material in the blood, which helps to identify mutations and chemical alterations linked with cancer. A key element of liquid biopsy, ctDNA carries primary tumor-representative genetic alterations and epigenetic changes. This makes it possible to find cancer-specific changes including mutations in TP53, KRAS, and EGFR genes (Diaz & Bardelli, 2014; Wan et al., 2017). Though less common, CTCs provide insightful analysis of tumor heterogeneity and metastatic potential (Alix-Panabières & Pantel, 2013). Furthermore enhancing the diagnostic value of liquid biopsies is the tiny vesicles released by cancer cells carrying proteins and nucleic acids reflecting the molecular profile of the tumor (Valadi et al., 2007). Early diagnosis of some tumors has shown great promise for liquid biopsy, especially when combined with imaging or other screening tools. For example, ctDNA detection helps to identify actionable mutations, such as EGFR mutations, in lung cancer before the disease becomes symptomatic, hence increasing the success rate of targeted therapy (Yu et al., 2013). Liquid biopsy has been used to find minimal residual disease following surgery in colorectal cancer, therefore offering early warnings of recurrence long before clinical symptoms show (Diehl et al., 2008). Likewise, with breast cancer, liquid biopsy can identify mutations in PIK3CA and BRCA1/2, thereby enabling individualized treatment plans catered to the genetic composition of the patient (Dawson et al., 2013). Liquid biopsy offers one of the most important benefits since it is

noninvasive, so lowering the danger and discomfort connected with tissue samples. Patients with difficult-to-access tumors, such as brain or lung malignancies, where conventional biopsies carry more danger, also find great benefit from it. Moreover, liquid biopsy makes it possible to track tumor development serially, thereby helping to identify newly occurring mutations perhaps resistant to treatment. This real-time surveillance helps to quickly modify therapy plans, hence maximizing patient results (Beaver et al., 2014; Oxnard et al., 2014). Liquid biopsy has several restrictions even if it offers certain benefits. Because tumor DNA in the blood is low, ctDNA's detection sensitivity especially in early-stage cancers remains a difficulty. Though instructive, CTCs are very scarce and challenging to consistently separate (Crowley et al., 2013; Parkinson et al., 2012). Furthermore, the problem is the absence of uniform liquid biopsy procedures for several cancer types. Although technological developments are enhancing these features, liquid biopsy is now a complementary tool rather than a universal replacement for tissue biopsy in all circumstances.

9.7 Biomarker-based multicancer early detection

9.7.1 The concept and potential of MCED

MCED is a new field that uses a single, noninvasive test to find several types of cancer at the same time. Biomarker-based MCED finds cancers by looking for ctDNA and other molecular markers in body fluids, especially blood, a long time before any signs show up. Usually, cancer screening methods focus on one type of cancer at a time, like mammograms for breast cancer or colonoscopy for colorectal cancer (Bettegowda et al., 2014). This plan is very different from those methods. MCED is based on looking at circulating cancer DNA (ctDNA), which has mutations, methylation patterns, and other changes that are unique to tumors. These molecular changes act as cancer fingerprints, enabling the single-test identification of several malignancies. Along with epigenetic markers like DNA methylation patterns, MCED uses mutations in genes like TP53, KRAS, and PIK3CA as among the main biomarkers. Particularly the methylation pattern has been demonstrated to be a quite sensitive biomarker for differentiating malignant from non-cancerous cells (Diaz & Bardelli, 2014; Wan et al., 2017). Many MCED tests have lately been developed. Among the most well-known is the Galleri test, which analyzes DNA methylation patterns in ctDNA to identify over 50 varieties of cancer from a blood test. Studies have revealed that this test can find difficult-to-detect tumors including pancreatic and ovarian cancer, which do not yet have efficient screening techniques (Klein et al., 2021).

9.7.2 Challenges and future directions of MCED

MCED tests hold the promise of early detection in cancers that are often diagnosed at advanced stages, thus improving overall survival rates (Oxnard et al., 2014).

Early diagnosis of tumors commonly detected at advanced stages is promised by MCED tests, therefore enhancing general survival rates (Crosby et al., 2022). Since MCED permits early diagnosis in asymptomatic people over a wide spectrum of malignancies, its clinical use is transforming. Particularly for cancers not covered by present screening systems, early detection has the potential to greatly enhance treatment outcomes. For example, MCED (Cohen et al., 2018), can help to identify early stages of cancers like ovarian, liver, and pancreatic cancers, which have significant death rates and are typically discovered at late stages. In populations at high risk of several malignancies, including those with inherited cancer syndromes like Lynch syndrome or BRCA1/2 mutations, MCED is also quite important. Although conventional screening approaches might not cover all possible cancer sites for these patients, an MCED test offers thorough surveillance (Diehl et al., 2008). Moreover, the noninvasive character of these tests helps patients to accept them better thereby improving compliance with cancer screening advice. Though MCED has great promise, some difficulties still exist. The necessity of raising the specificity of these tests to lower false positives represents one of the main constraints. False positives could result in unwarranted diagnostic tests, increasing stress, and maybe intrusive follow-ups (Crowley et al., 2013). Another problem is that ctDNA can look different in different types of cancer and at different stages. This could make the test less sensitive in early-stage cancers where ctDNA amounts are low (Alix-Panabières & Pantel, 2013). MCED study projects in the future will focus on making biomarker panels better so that they are more sensitive and specific. Improving the diagnostic accuracy of MCED tests by combining genomes, proteomics, and metabolomics data using multi-omics techniques that is, Furthermore improving the predictive ability of these tests would be the analysis of vast biomarker databases using artificial intelligence and machine learning algorithms (Parkinson et al., 2012).

9.8 Conclusion

The identification and practical use of cancer biomarkers have revolutionized the field of oncology by creating new avenues for individualized care, early identification, and diagnosis. Thanks to developments in genomes, proteomics, and metabolomics, diagnostic biomarkers have become powerful tools for early cancer identification, often before symptoms appear. Consequently, patient outcomes have been significantly enhanced. The transformative potential of these biomarkers in clinical practice is underscored by case studies, notably in prostate, ovarian, breast, and lung cancers. Nevertheless, there are still obstacles to overcome, particularly in the areas of optimizing the sensitivity and specificity of biomarkers and addressing the biological heterogeneity of tumors. To overcome these obstacles, the field of cancer research must incorporate cutting-edge technology such as liquid biopsy, multi-omics techniques, and artificial

intelligence. With further research and development, biomarker-driven screening programs have the potential to improve cancer treatment accuracy and reduce overdiagnosis while also revolutionizing the way early cancer detection is currently done. Biomarkers are essential for personalized treatment plans, which is why oncology is expected to prioritize personalized medication in the future. By customizing therapy procedures to the individual molecular profile of each patient, the medical community can offer more effective and less hazardous pharmaceuticals, thereby enhancing the quality of life and survival rates of cancer patients worldwide. The ongoing research on biomarker identification and its clinical application is establishing a new era of patient-centered, noninvasive, and precise cancer treatment by bridging the divide between the laboratory and the clinic.

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CHAPTER

Prognostic biomarkers: predicting disease outcomes

10

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10.1 Introduction

A biomarker is a biomolecule, used to identify the likelihood of a clinical event, disease recurrence, or progression in patients who have the disease or medical condition of interest. A prognostic biomarker implies a higher (or lower) probability of a future clinical event, illness recurrence, or progression. The measurement of prognostic biomarkers occurs at a predetermined baseline, which could involve background therapy. Prognostic biomarkers are commonly observed in clinical settings where a patient has received a diagnosis and there is a desire to determine the probability of a subsequent clinical event. Future events can include things like dying, getting sicker, getting sicker again, or getting sicker again. Biomarkers for prognosis in oncology have historically included tumor size, the number of lymph nodes positive for tumor cells, and the occurrence of metastases. Molecular signatures or indicators assessed on tumors are increasingly being used in place of or in addition to these clinicopathologic features. Biomarkers that indicate an increased risk for future heart attack include low high density lipoprotein (HDL) cholesterol, raised blood pressure, indications of diabetes, and elevated low density lipoprotein (LDL) cholesterol in patients who have already experienced a heart attack. Concomitant evidence of diabetes is linked to a higher risk of cardiovascular events in those with hypertension. The relationship between the prognostic biomarker and outcome (i.e., predicts a higher likelihood of an occurrence without an intervention) exists independently of other therapies. However, the precise clinical situation (e.g., background therapy, disease stage) and specific endpoint of interest may affect the presence or strength of a predictive correlation, so prognostic biomarkers must be presented in the appropriate context (Murad & Melamud, 2022).

Prognostic signs are commonly used as eligibility criteria in clinical trials to identify patients who are more likely to encounter clinical events or see their disease progress. They are therefore widely used as enrichment factors in drug development. The endpoint of many clinical studies, including those involving medicinal

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interventions, is an incidence rate or time-to-event. The statistical power of a time-to-event endpoint to assess treatment impact in a controlled clinical trial is determined by the intended effect magnitude (i.e., hazard ratio) and the anticipated number of occurrences. Consequently, incorporating patients who have a higher probability of experiencing an event will enhance and increase statistical power. This scenario is analogous to the use of susceptibility/risk biomarkers to enrich populations in preventative trials. Prognostic biomarkers can help determine whether and how strongly to intervene with treatment in a therapeutic environment.

Within the biomedical field, the term "prognostic" has not always been applied consistently. Some have limited the use of the phrase to those with a diagnosis of an illness or other medical condition in a clinical setting. Prognostic biomarkers would also include those that predict the chance of a future diagnosis or illness in otherwise healthy individuals. This chapter distinguishes between susceptibility/risk biomarkers, which are defined as those that apply to persons who do not exhibit a clinically obvious disease, and prognostic biomarkers (Murad & Melamud, 2022).

Prognostic biomarkers are measurements that are utilized in clinical diagnostic procedures to provide information about a patient's prognosis. For patients who are either receiving standard care or have not had any therapy at all, these indicators, which are usually evaluated before treatment, should demonstrate long-term outcomes. The purpose of its application is to indicate the probability of a clinical outcome, such as the onset or progression of an illness. It is important to distinguish susceptibility/risk biomarkers, which are focused on connections with the transition from a state of health to disease, from prognostic biomarkers, even though this distinction is not widely recognized. Additionally, they differ from predictive biomarkers, which identify characteristics associated with the result of an exposure or action. There are several biomarkers for autoimmune diseases that provide prognostic information. For example, elevated anti-CarP antibodies in rheumatoid arthritis (RA) patients indicate the likelihood of developing erosive illness later on (Melus et al., 2021). Molecular events that predict the course of a disease are known as prognostic biomarkers. Various prognostic readouts are used, depending on the condition. The most generally used applications are progression-free survival, where a patient does not worsen, and survival rate/time, which indicates the percentage of patients that survive after a given period. These indications are ultimately intended to better define patients biologically so that treatments using precision medicine can be delivered more effectively (Hlady & Robertson, 2016).

Prognostic biomarkers are biological traits of a tumor that can help determine whether individuals require more rigorous therapy and may reveal information about the overall course of the illness. However, prognostic indicators are unable to predict how a patient will respond to a certain treatment. Predictive biomarkers are traits that indicate how likely a treatment is to be successful. Much like with many other types of cancers, many potential biomarkers are discovered, but only a small number are validated in an independent group of patients. Prognostic biomarkers must both receive independent validation and outperform established indicators like as stage, age, and performance status to be relevant in clinical practice.

Predictive biomarkers need further validation in biomarker-driven prospective clinical studies. There are currently no validated companion predictive biomarkers being utilized in clinical settings in muscle invasive bladder cancer (MIBC), several prognostic and predictive biomarkers have been investigated; however, although some exhibit potential, none have obtained sufficient confirmation from prospective clinical studies to be employed as standard practice at this time. As was already noted, biomarkers can direct treatment choices in several areas of MIBC, avoiding unnecessary toxicity and improving survival and quality of life outcomes (Wilson et al., 2022).

The development of prognostic biomarkers that can provide vital information about the course of the disease in children, such as the emergence of allergic asthma and the possibility of remission before adolescence or continuous ongoing chronic inflammation, is another unmet need that needs to be addressed. Moreover, it may be possible to identify a predictor for severe viral side effects such as eczema herpeticum. We have discovered that atopic dermatitis (AD) may be a chronic illness with periods of low activity and later reactivation. Thus prognostic biomarkers that can forecast this stage of life may be highly helpful in preventing AD and other potential comorbidities in elderly adults (Bieber et al., 2017).

A companion diagnostic biomarker, also known as a predictive biomarker, can be used to identify patients who are most likely to benefit from a therapeutic intervention; it can also be used to identify patients who may be more susceptible to serious adverse reactions as a result of receiving the drug; and it can be used to monitor the patient's response to the drug's treatment to make necessary adjustments to the schedule, dosage, or duration of the medication to improve safety or efficacy. The purpose of utilizing a prognostic biomarker, which might be useful in the process of selecting patients for therapy, is to objectively evaluate the patient's overall result. Classifying patients and determining which ones are most likely to benefit from a medicine or therapy should be made easier with its assistance (Egger & Arimondo, 2016; Sheikh et al., 2016).

Physicians may find it easier to determine whether systemic chemotherapy, local radiation therapy, or surgical excision is required with the aid of predictive biomarkers. Currently, CA 19-9 is only partially helpful in making these kinds of decisions, and other biomarkers like CEA and CA-125 that are commonly used in pancreatic cancer surveillance have been hampered by their low disease specificity. Proteins and genetic markers are other biomarkers that have garnered attention as possible prognostic indicators for pancreatic cancer; however, none of these have yet to provide enough evidence to be employed in clinical settings. Numerous proteins are potential predictors of the prognosis of pancreatic cancer. Three of these proteins—MUC1, MUC2, and mesothelin among 13 proteins linked to pancreatic cancer—were discovered to be potential markers that could differentiate the disease based on tumor aggressiveness. The altered glycosylation patterns of membrane-bound proteins MUC1 and MUC2 are connected to several cancer types. Mesothelin is a glycosylphosphatidylinositol-anchored protein present on the cell surface that is overexpressed in mesothelioma, ovarian cancer, and squamous cell

carcinoma. Osteopontin (OPN), another protein biomarker that has shown promise in the early identification of the disease, has also been linked as a prognostic biomarker based on the finding that blood levels of OPN in patients with pancreatic cancer are associated with a worse chance of survival. Human antigen R (HuR), an RNA-binding protein, has a role in regulating gene expression in response to cellular stress. Numerous intra- and extracellular processes require the enzyme tissue transglutaminase II (TgII). It acts as a catalyst in transamidation reactions that require calcium. It has been demonstrated that TgII contributes to drug resistance, cell motility and migration, and the development and spread of cancer. Many studies are currently being conducted on TgII as a prognostic and therapeutic target for pancreatic cancer (Nolen & Lokshin, 2014; Rastogi et al., 2016).

Prognostic biomarkers are very useful and practical in the clinical arena since they allow one to plan for the future clinical course of a disease. Indicators of disease progression can be used as longitudinal assessments to track motor neuron loss or functional ability in **survival motor neuron (**SMA) patients objectively over time. Prognostic and disease progression biomarkers can be validated without effective medicines, and treatment does not necessarily alter these biomarkers (Arnold et al., 2017; Navarrete-Opazo et al., 2021).

Prognostic biomarkers are very important for predicting an individual's chance of an event or an adverse outcome. Using this information will help you decide how long to stay in a hospital or intensive care unit. By stratifying the risk for both unfavorable clinical and financial outcomes, predictive biomarkers are also widely used in population health resource allocation, allowing a healthcare organization to identify patients who would benefit from more thorough evaluation while sparing others from unnecessary additional testing or medical interventions (Califf, 2018).

The preliminary treatment measurements of a tumor's molecular or histopathological features, such as germline or somatic mutations, alterations in DNA methylation, micro-RNA levels, or circulating tumor cells in the blood, that are linked to the long-term course or outcome of disease are known as prognostic markers. Survival in patients expressing the relevant biomarker is comparable in those receiving treatment versus those not receiving it. It is possible to identify patients who require adjuvant therapy or more stringent surveillance thanks to predictive biomarkers. Cytogenetic abnormalities are used as prognostic indicators for risk classification in acute myeloid leukemia. Chromosomal inversions in chromosome 16 and translocations between chromosomes 8 and 21, and 15 and 17 are linked to a better prognosis, whereas chromosome 5 and 7 deletions are linked to a worse prognosis. Before starting systemic therapy for multiple myeloma, levels of beta-2 microglobulin and albumin are employed as prognostic markers to stage the disease and assign patients to favorable, intermediate, or unfavorable overall survival prognoses. Prognostic indicators for breast cancer can include tumor size, grade, nodal status, and the presence or absence of lymphovascular invasion (Pezo and Bedard, 2015).

Identifying predictive biomarkers provides information on the likelihood of responding to a specific therapy and are typically evaluated before treatment.

For patients who display the biomarker linked to a certain therapy's response, the likelihood of survival is dependent on treatment. There is no difference in survival between treated and untreated patients who test negative for biomarkers. The expression of the HER2/neu protein is one instance of a predictive biomarker in breast cancer. The HER2/neu protein, which belongs to the epidermal growth factor receptor (EGFR) family of transmembrane receptors, is expressed more often in 15%–20% of patients with invasive breast cancer. This increased expression is linked to how these patients respond to anti-HER2-targeted medications including trastuzumab, pertuzumab, and trastuzumab–emtansine. Another instance is the correlation between the expression of the estrogen receptor and the sensitivity to hormonal drugs utilized in adjuvant and metastatic contexts in breast cancer (Pezo and Bedard, 2015).

Prognostic biomarkers can be broadly categorized into two types based on their predictive value: those associated with favorable outcomes and those linked to poor prognoses. Biomarkers associated with good prognoses are indicative of a positive response to treatment, relatively slower disease progression, or increased overall survival rates. These biomarkers offer invaluable insight for tailoring personalized treatment plans and optimizing patient care. Conversely, biomarkers associated with poor prognoses signify a higher risk of disease progression, treatment resistance, or reduced survival rates, highlighting the necessity for intensified interventions and close monitoring.

The types of prognostic biomarkers associated with good prognosis encompass various biological indicators, including specific genetic mutations, favorable gene expression profiles, and the presence of certain proteins or receptors. For instance, in cancer research, the expression of estrogen receptors in breast cancer is considered a favorable prognostic biomarker. Similarly, genetic mutations such as the presence of certain alleles in leukemia patients can indicate a better response to specific treatments, ultimately leading to improved prognoses. Good prognostic Biomarkers provide actionable information that can guide treatment decisions and improve patient outcomes. KRAS mutations are commonly assessed in colorectal cancer. Patients with KRAS mutations may not benefit from EGFR-targeted therapies, which helps in tailoring treatment plans. HER2-positive breast cancer is associated with more aggressive disease but also indicates that HER2-targeted therapies like trastuzumab can be highly effective (Catalona et al., 1991; Slamon et al., 1987; Strickler et al., 2023).

Conversely, prognostic biomarkers indicative of poor prognoses encompass factors such as high levels of specific enzymes or proteins, genetic abnormalities associated with aggressive disease behavior, or tumor microenvironment characteristics that support disease progression. Elevated levels of certain biomarkers like CA-125 in ovarian cancer patients often correlate with advanced disease stages and poor prognoses. Additionally, genetic mutations associated with resistance to conventional treatments signify a challenging clinical course and a less favorable prognosis. Bad prognostic biomarkers might indicate poor prognosis but may not provide specific or actionable information for treatment decisions. CA-125 levels

can be elevated in ovarian cancer but also other conditions. High levels can indicate a poor prognosis, but it is not specific enough for reliable diagnosis or monitoring alone. Elevated levels of LDH can indicate aggressive disease and poor prognosis in various cancers, but it lacks specificity and can be elevated in a range of other conditions (Bast et al., 1983; Chen & Zou, 2023).

Understanding the intricate interplay between these prognostic biomarkers and disease outcomes is imperative for advancing clinical decision-making and improving patient management strategies. Moreover, the integration of advanced technologies such as next-generation sequencing and multiplex protein assays has revolutionized the identification and utilization of prognostic biomarkers, enabling more precise prognostic predictions in clinical settings. The integration of artificial intelligence and machine learning algorithms further enhances the predictive power of these biomarkers, paving the way for enhanced prognostic accuracy and personalized treatment strategies.

In conclusion, the delineation of prognostic biomarkers into categories of good and bad prognoses is instrumental in guiding clinical decision-making and optimizing patient care. The ongoing advancements in biomarker discovery and the integration of cutting-edge technologies continue to reshape the landscape of prognostic predictions, ultimately fostering improved patient outcomes and transforming the paradigm of precision medicine. A comprehensive understanding of prognostic biomarkers is pivotal in driving future advancements and innovations in the realm of predictive medicine (Table 10.1).

Table 10.1 Some good and bad prognostic biomarkers of diseases.

S no.	Cancer	Prognostic biomarkers marker	Nature of P biomarkers	References
1	Pancreatic ductal adenocarcinoma (PDAC)	Serum osteopontin (OPN), Tissue inhibitor of metalloproteinase 1 (TIMP-1)		Poruk et al. (2013)
2	Colorectal cancer	KRAS mutation	Genetic	Strickler et al. (2023)
3	Breast cancer	HER2	Protein	Slamon et al. (1987)
4	Prostate cancer	Prostate-specific antigen	Protein	Catalona et al. (1991)
5	Small cell lung cancer	EGFR	Genetic	Park et al. (2016)
6	Breast cancer	CDK4,	Genetic	Mishra et al. (2023)
7	Breast cancer	PTEN	Genetic	Mishra et al. (2023)

(*Continued*)

8	HCC	MALAT1	Genetic	Mishra et al. (2023)
9	HCC & GBM	differentially expressed genes (DEGs)		Mishra et al. (2023)
10	Bladder urothelial carcinoma (BLCA)	TCGA	Genetic	Chatterjee et al. (2024)
12	Bladder cancer	Poly C Binding Protein 1 (PCBP1)	Protein	Luo et al. (2023)
13	Breast cancer	LIM and senescent cell antigen-like-containing domain protein 1 (LIMS1)	Protein	Li et al. (2023)
14	Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC)	Heterogeneous nuclear ribonucleoprotein A1 (HNRNPA2B)	Protein	Dong et al. (2023)
15	Colon adenocarcinoma	Signal recognition particle 72	Protein	Kirwan et al. (2012)
16	Angina/Acute myocardial infarction/ coronary artery disease	C-reactive protein	Protein	Pearson et al. (2003)
17	Chronic obstructive pulmonary disease	Plasma fibrinogen	Protein	Miller et al. (2016)
18	Chronic lymphocytic leukemia	Chromosome 17p deletions and TP53 mutations	Genetic	Gonzalez et al. (2011)
19	Prostate cancer	Gleason score		Epstein et al. (2016), Gordetsky & Epstein (2016, 2016)
20	Autosomal dominant polycystic kidney disease	Total kidney volume		Grantham et al. (2006)
	Ovarian cancer	CA-125	Protein biomarker	Bast et al. (1983).
	Cancers	Lactate dehydrogenase	Enzyme	Liao (2016)

10.2 Prognostication

Prognostication is the process of predicting the future course and outcome of a disease. It plays a crucial role in clinical decision-making by providing insights into disease progression, potential complications, and overall survival. Accurate prognostication

can guide treatment decisions, patient counseling, and resource allocation. It involves predicting the likely outcome of a disease based on various factors. This process is essential for personalizing treatment plans, optimizing patient management, and improving quality of life. It helps clinicians to tailor interventions based on an individual's risk profile and expected disease trajectory.

Prognostic factors can be broadly categorized into:

- **Clinical Factors**: Patient's age, overall health, comorbidities, and performance status.
- **Pathological Factors**: Tumor type, grade, stage, and histological characteristics.
- **Biological Markers**: Genetic mutations, protein levels, and other molecular markers.

10.2.1 Methods of prognostication

10.2.1.1 Risk models and scoring systems

Risk models integrate multiple prognostic factors to estimate the probability of specific outcomes. These models often use statistical techniques to combine variables and generate a risk score. The *Gleason Score* in prostate cancer assesses the aggressiveness of cancer based on histological patterns. Higher scores indicate a worse prognosis and guide treatment decisions (Epstein, 2005).

10.2.1.2 Biomarkers

Biomarkers are biological molecules that provide information about the disease. They can be proteins, genes, or other substances that reflect disease status. *Prostate-specific antigen (PSA)* is used in prostate cancer to gauge disease progression and response to treatment. Elevated levels suggest advanced or recurring disease (Catalona et al., 1991).

10.2.1.3 Imaging techniques

Imaging techniques provide visual information about the extent of the disease and help in monitoring the progression or response to treatment. *PET/CT Scans* in oncology assess the metabolic activity of tumors. Increased uptake of radiotracers indicates higher tumor activity and can predict prognosis (Juweid & et al., 2007).

10.2.2 Prognostication in different diseases

Predicting the prognosis of a disease requires a comprehensive understanding of the pathophysiology, natural history, and predictive factors associated with the condition. Advances in medical research have led to the development of sophisticated prognostic models and scoring systems that are tailored to specific diseases. These tools are invaluable for healthcare professionals involved in patient care and clinical

research, allowing them to make evidence-based predictions regarding disease progression, mortality, and response to treatment.

10.2.2.1 Cardiovascular diseases

Prognostication is integral to the management of chronic diseases such as heart failure. Risk prediction scores like the Seattle Heart Failure Model enable healthcare providers to assess the likelihood of adverse cardiovascular events and mortality in patients with heart failure, facilitating personalized management and end-of-life discussions. Additionally, prognostic markers such as brain natriuretic peptide levels serve as valuable indicators of disease severity and prognosis in this patient population. Prognostication in cardiovascular diseases often involves evaluating risk factors such as blood pressure, cholesterol levels, and genetic predispositions. The *Framingham Risk Score* is a widely used tool to estimate the 10-year risk of cardiovascular events based on factors like age, sex, smoking status, and blood pressure (D'Agostino et al., 2008).

10.2.2.2 Cancer

In the field of oncology, prognostication plays a critical role in guiding treatment decisions and counseling patients. For instance, in breast cancer, the use of tools such as the Nottingham Prognostic Index helps healthcare professionals estimate the risk of disease recurrence and overall survival based on tumor characteristics. Similarly, in hematological malignancies, risk stratification models such as the International Prognostic Scoring System aid in predicting the prognosis of conditions like myelodysplastic syndromes, informing the selection of appropriate treatment strategies. Cancer prognostication is complex due to the heterogeneity of the disease. It involves evaluating tumor characteristics, patient factors, and molecular markers. In breast cancer, the *Oncotype DX* test analyzes the expression of a panel of genes to predict the likelihood of recurrence and benefit from chemotherapy (Paik et al., 2004).

10.2.2.3 Neurological diseases

Prognostication in neurological diseases often relies on clinical features and imaging studies to estimate disease progression and outcomes. In Alzheimer disease, *cerebrospinal fluid (CSF) biomarkers* such as amyloid-beta and tau proteins are used to predict disease progression and cognitive decline (Blennow, 2010).

10.2.2.4 Infectious diseases

Infectious diseases also benefit from prognostication, particularly in the context of emerging infections and global health threats. For instance, during the COVID-19 pandemic, healthcare professionals utilized clinical scoring systems and biomarkers to assess the prognosis of infected individuals, aiding in resource allocation and treatment prioritization. Prognostication in infectious diseases involves predicting the likely course and outcome of an infection based on various factors, including

clinical, laboratory, and epidemiological data. As of the latest information, here are some key points and trends in prognostication for infectious diseases:

10.2.2.4.1 Sepsis and septic shock

Recent advances focus on biomarkers like Procalcitonin (PCT) and C-reactive protein for prognostication. Elevated levels of PCT, in particular, are linked to more severe infections and worse outcomes. New predictive models using machine learning algorithms have been developed to better forecast outcomes based on a wide array of data, including electronic health records (EHRs) and laboratory tests (Seymour, 2024; Aygun et al., 2024).

10.2.2.4.2 COVID-19

Research has identified several risk factors for severe outcomes, including age, comorbidities (such as diabetes and cardiovascular disease), and biomarkers like D-dimer and IL-6. Ongoing studies aim to understand the long-term effects and prognostic factors for patients experiencing prolonged symptoms after acute COVID-19 infection (Verity, 2024; Xie et al., 2024).

10.2.2.4.3 Tuberculosis

Advances in genomic sequencing and molecular diagnostics are helping to predict the likelihood of drug resistance and treatment outcomes. Research is increasingly focusing on genetic and immune system factors that influence tuberculosis progression and treatment response (Al-Khodor, 2024; Yin et al., 2024).

10.2.2.4.4 HIV/AIDS

The prognosis of HIV/AIDS is heavily influenced by viral load and CD4 count. Recent studies emphasize the importance of early ART (antiretroviral therapy) initiation to improve long-term outcomes. The use of drug resistance testing has become a key component in tailoring effective treatment regimens (Gupta, 2024a, 2024b).

10.2.2.4.5 Antimicrobial resistance

There is growing interest in predictive modeling to forecast the impact of antimicrobial resistanceon infection outcomes. These models consider factors like resistance patterns and the effectiveness of current treatment options (Michael, 2024).

10.2.2.4.6 Fungal infections

New molecular diagnostic tools are improving the ability to predict the outcomes of fungal infections, such as candidiasis and aspergillosis, based on the species and resistance patterns. Molecular Diagnostic Approaches for Fungal Infections." Infection and Immunity (Pappas, 2024).

10.2.3 Developments in prognostication

10.2.3.1 Integrating artificial intelligence

Integrating artificial intelligence (AI) into prognostication for infectious diseases is an area of rapid development. Recent advancements in AI and machine learning have enhanced prognostication by analyzing large datasets to identify patterns and predict outcomes with high accuracy. AI algorithms applied to medical imaging and EHRs can now predict patient outcomes and treatment responses with unprecedented precision. (Esteva et al., 2019; Gupta, 2024a, 2024b; Liu, 2024; Patel, 2024; Wang, 2024a, 2024b; Zhang, 2024a, 2024b)

10.2.3.1.1 Predictive modeling

AI algorithms can analyze large datasets from EHRs to identify patterns and predict patient outcomes. For example, machine learning models can predict sepsis risk by analyzing vital signs and lab results. AI can forecast the progression of infectious diseases by integrating data from various sources, including patient demographics, clinical parameters, and laboratory results.

10.2.3.1.2 Personalized treatment

AI models can assist in developing personalized treatment plans based on individual patient data, improving the effectiveness of interventions and minimizing adverse effects. In diseases like tuberculosis and HIV, AI can predict drug resistance by analyzing genetic data and resistance patterns, enabling more effective treatment choices.

10.2.3.1.3 Early detection and monitoring

AI can enhance early detection systems by analyzing real-time data and identifying potential outbreaks or severe cases before they escalate. AI-driven tools can monitor patient data continuously, providing timely alerts for changes in condition that may indicate worsening disease.

10.2.3.1.4 Data integration and analysis

AI can process and integrate vast amounts of data from diverse sources (e.g., clinical, genomic, and environmental data) to provide comprehensive prognostic insights. Natural language processing (NLP) algorithms can extract relevant information from unstructured clinical notes, improving the accuracy and completeness of prognostic models.

10.2.3.2 Liquid biopsies

Liquid biopsies are a revolutionary advancement in the field of medical diagnostics and prognostication. They involve analyzing biomarkers from bodily fluids (such as blood, urine, or CSF) to gain insights into disease presence, progression, and response to treatment. This approach is especially valuable in infectious diseases,

oncology, and chronic conditions. Liquid biopsies, which analyze circulating tumor DNA (ctDNA) in blood samples, offer a noninvasive method for monitoring disease progression and response to treatment. Liquid biopsies represent a significant advancement in prognostication and personalized medicine. They offer a less invasive method for monitoring disease progression, detecting biomarkers, and personalizing treatment strategies. The latest research continues to explore and expand the applications of liquid biopsies, improving their accuracy and utility in various medical fields.

10.2.3.3 Role of liquid biopsies in prognostication

10.2.3.3.1 Cancer detection and monitoring

Liquid biopsies can detect ctDNA, which can be used to monitor tumor dynamics, detect minimal residual disease, and predict relapse or response to therapy. They offer a non-invasive method for early cancer detection, potentially improving outcomes through earlier intervention. In lung cancer, ctDNA testing can detect minimal residual disease and predict relapse earlier than conventional imaging (Hironaka-Mitsuhashi et al., 2019).

10.2.3.3.2 Infectious diseases

Liquid biopsies can identify pathogens and their genetic material in blood or other fluids, aiding in early diagnosis and monitoring of infections. They can help detect resistance genes and mutations, guiding more effective treatment choices (Ray & Vohra, 2022).

10.2.3.3.3 Personalized medicine

Liquid biopsies facilitate the identification of biomarkers associated with disease prognosis and treatment response, enabling personalized treatment strategies. They provide a method for continuous monitoring of disease progression and treatment efficacy, allowing for therapy adjustments based on real-time data (Ray & Vohra, 2022).

10.2.3.3.4 Noninvasive monitoring

As a non-invasive alternative to tissue biopsies, liquid biopsies reduce the risk and discomfort associated with traditional biopsy methods and are suitable for repeated testing (Ma et al., 2024).

10.2.3.4 Multi-omics approaches

Multi-omics approaches in prognostication involve integrating various types of omics data such as genomics, transcriptomics, proteomics, metabolomics, and epigenomics to provide a comprehensive view of disease mechanisms, progression, and patient outcomes. These approaches enable a more holistic understanding of complex diseases and enhance the accuracy of prognostic models.

Combining data from genomics, proteomics, and metabolomics (multi-omics) provides a comprehensive view of disease and enhances prognostication. Multi-omics approaches in cancer research integrate genetic, transcriptomic, and proteomic data to identify novel biomarkers and predict patient outcomes more accurately (Mohr et al., 2024; Menyhárt & Győrffy, 2021; Ozaki et al., 2024).

10.2.3.5 Role of multi-omics approaches in prognostication

10.2.3.5.1 Comprehensive disease profiling

Integration of data types: Multi-omics integrates data from different omic layers, such as DNA sequences (genomics), RNA expression profiles (transcriptomics), protein levels (proteomics), and metabolite concentrations (metabolomics), providing a more complete picture of the biological state of a disease. Disease mechanisms: By combining these data types, researchers can identify complex interactions and pathways involved in disease development and progression, which are often missed when analyzing single omic layers alone (Ozaki et al., 2024).

10.2.3.5.2 Personalized medicine

Multi-omics approaches enable the identification of specific biomarkers and disease subtypes, facilitating the development of personalized treatment plans based on individual molecular profiles. Integration of multi-omics data improves the accuracy of predictive models for disease outcomes and treatment responses, allowing for more precise and individualized interventions (Wang et al., 2023).

10.2.3.5.3 Early detection and diagnosis

Multi-omics can reveal novel biomarkers and signatures that are indicative of disease onset, progression, or response to treatment, leading to earlier and more accurate diagnoses. By integrating data across multiple omics platforms, researchers can better classify diseases into subtypes, which can be crucial for early detection and targeted treatment (Chen et al., 2023).

10.2.3.5.4 Monitoring and prognosis

Continuous monitoring of patients using multi-omics approaches can provide insights into disease progression and treatment efficacy, enabling timely adjustments in therapy. Multi-omics data integration enhances the ability to predict patient outcomes by considering a wide range of molecular information, leading to more robust prognostic models (Carraro et al., 2024; Eicher et al., 2020; Wang, 2024a; Wang, 2024b).

Prognostication is a dynamic and evolving field that integrates various methods and technologies to predict disease outcomes. The integration of risk models, biomarkers, imaging techniques, and emerging technologies like AI and liquid biopsies has significantly improved the accuracy and utility of prognostic

assessments. Continued advancements in these areas promise to enhance personalized medicine, optimize treatment strategies, and ultimately improve patient outcomes.

Multi-omics approaches provide a powerful tool for disease prognostication by integrating diverse molecular data to offer a comprehensive view of disease states and progression. The latest research highlights the growing importance of these approaches in personalized medicine, early detection, and monitoring across various medical fields. The continuous advancements in omics technologies and data integration methodologies are expected to further enhance prognostic accuracy and treatment efficacy.

10.3 Classification

Prognostic biomarkers are crucial in predicting the progression and outcome of diseases, guiding treatment decisions, and improving patient management. The classification of prognostic biomarkers can be based on their nature, including genetic, epigenetic, protein, and imaging biomarkers. This classification helps in understanding how different types of biomarkers contribute to prognostication and their applications in clinical practice. Here, we will explore each category in detail, providing recent examples and references.

10.3.1 Genetic prognostic biomarkers

In the rapidly evolving landscape of precision medicine, genetic prognostic biomarkers play a pivotal role in predicting disease outcomes, guiding treatment decisions, and ultimately improving patient care. For students, healthcare professionals, and scientists understanding the latest developments in this field is instrumental for advancing research and clinical practice. Genetic prognostic biomarkers encompass a wide range of genetic variations, including single nucleotide polymorphisms (SNPs), copy number variations, epigenetic modifications, and gene expression profiles. These biomarkers provide valuable insights into an individual's susceptibility to diseases, disease progression, and response to specific treatments. Notably, the continuous advancements in genomic technologies have enabled the identification of increasingly specific and reliable prognostic biomarkers, revolutionizing the approach to personalized medicine.

One of the key aspects of genetic prognostic biomarkers is their potential to predict disease outcomes with a high level of accuracy. By analyzing an individual's genetic profile, healthcare professionals and researchers can stratify patients based on their risk of developing certain conditions, such as cancer, cardiovascular diseases, and neurological disorders. The information not only allows for early intervention and targeted monitoring but also holds promise for the development of tailored therapeutic strategies.

Moreover, genetic prognostic biomarkers are invaluable for optimizing treatment selection and dosing, thereby minimizing the risk of adverse effects and enhancing treatment efficacy. For instance, in oncology, the identification of predictive genetic biomarkers has enabled the implementation of targeted therapies that specifically address the molecular characteristics of a tumor, leading to notable improvements in patient outcomes and survival rates.

The significance of staying updated on the latest references in genetic prognostic biomarkers cannot be overstated. Recent studies have uncovered novel biomarkers associated with diverse diseases, shedding light on previously unrecognized factors that influence disease prognosis. Additionally, the integration of multi-omics data, including genomics, transcriptomics, proteomics, and metabolomics, has expanded our understanding of the complex interplay between genetic factors and disease progression, paving the way for more comprehensive prognostic models.

One notable example of the evolving landscape of genetic prognostic biomarkers is the emergence of liquid biopsies as a noninvasive method for detecting and monitoring cancer through the analysis of circulating tumor DNA and other biomarkers. The potential of liquid biopsies to provide real-time insights into tumor dynamics and treatment response represents a groundbreaking development in cancer care, with implications for prognosis and treatment adaptation.

Genetic prognostic biomarkers are pivotal tools in the era of precision medicine, offering unprecedented opportunities for personalized healthcare and tailored interventions. For healthcare professionals, and scientists, embracing the latest advancements in this field is essential for driving innovation, improving patient outcomes, and advancing the frontiers of medical knowledge. As we continue to unravel the intricate genetic underpinnings of disease, the integration of genetic prognostic biomarkers into clinical practice holds immense promise for shaping a more precise and effective approach to healthcare.

Genetic biomarkers are DNA-based markers that provide information about genetic variations associated with disease risk, progression, and response to treatment. They include SNPs, gene mutations, and gene expression profiles.

10.3.1.1 Single nucleotide polymorphisms

SNPs are the most common type of genetic variation among individuals. They can influence an individual's susceptibility to diseases and response to treatments. The *BRCA1 and BRCA2* genes are associated with an increased risk of breast and ovarian cancer. Mutations in these genes can significantly affect prognosis and guide preventive strategies (King et al., 2003; Kuchenbaecker, 2017).

10.3.1.2 Gene mutations

Specific mutations in genes can be associated with poor prognosis or response to therapy. In lung cancer, mutations in the *EGFR* gene are associated with a better response to tyrosine kinase inhibitors but also indicate a more aggressive form of cancer if not treated early (Lynch et al., 2004; Zhou, 2011).

10.3.1.3 Gene expression profiles

Gene expression profiling can identify patterns associated with disease outcomes. The *Oncotype DX* test assesses the expression of 21 genes in breast cancer and predicts the risk of recurrence and benefit from chemotherapy (Paik et al., 2004; Sparano et al., 2018).

10.3.2 Epigenetic biomarkers

In the rapidly advancing field of precision medicine, epigenetic biomarkers are emerging as powerful tools for understanding and predicting disease processes. For healthcare professionals and scientists, epigenetics presents a compelling area of study with far-reaching implications for personalized healthcare and disease management.

Epigenetic biomarkers are molecular modifications that occur on the DNA and histone proteins, regulating gene expression without altering the underlying genetic code. These modifications can be influenced by environmental factors such as diet, stress, and exposure to toxins, making them dynamic indicators of an individual's health status. For researchers and healthcare professionals, the study of epigenetic biomarkers offers a window into the interplay between genetic predisposition and environmental influences, providing valuable insights into disease susceptibility and progression.

The potential applications of epigenetic biomarkers are diverse and far-reaching. In cancer research, these biomarkers hold promise for early detection, prognosis, and treatment response prediction. By analyzing the epigenetic modifications associated with specific types of cancer, researchers can identify biomarker signatures that facilitate personalized cancer diagnosis and treatment selection. For healthcare professionals, this could mean more effective and tailored treatment strategies, ultimately improving patient outcomes.

Moreover, in the realm of neurological disorders, epigenetic biomarkers offer a promising avenue for understanding conditions such as Alzheimer disease, Parkinson's disease, and autism spectrum disorders. By unraveling the epigenetic patterns associated with these complex conditions, scientists and healthcare professionals can gain deeper insights into disease etiology and progression. This knowledge opens the door to developing targeted therapies that address the unique molecular signatures of each individual, moving us closer to personalized treatments for neurodegenerative and neurodevelopmental disorders.

The study of epigenetic biomarkers provides a rich landscape for exploratory research and innovation. Understanding the mechanisms behind epigenetic modifications and their implications for human health catalyzes the development of novel diagnostic tools and therapeutic interventions. As the field continues to expand, there is substantial opportunity for students to contribute to groundbreaking discoveries that could shape the future of healthcare.

Crucially, the study of epigenetic biomarkers necessitates an interdisciplinary approach, drawing on expertise from genetics, molecular biology, bioinformatics,

and clinical medicine. By uniting diverse fields of study, this area of research fosters collaboration between scientists and healthcare professionals, paving the way for holistic approaches to patient care and disease management.

In conclusion, epigenetic biomarkers represent a frontier in precision medicine, offering a glimpse into the intricate interplay between genetics, environment, and disease. For master students, PhD students, healthcare professionals, and scientists, the study of epigenetic biomarkers opens up a world of opportunities to drive innovation in personalized medicine, with far-reaching implications for improving human health. As research in this field continues to unfold, it holds the potential to revolutionize the way we understand and treat a wide array of diseases, ultimately bringing us closer to the vision of truly individualized healthcare. Epigenetic biomarkers involve changes in gene expression that do not involve alterations in the DNA sequence. These changes include DNA methylation, histone modification, and non-coding RNA expression.

10.3.2.1 DNA methylation

DNA methylation refers to the addition of methyl groups to DNA, affecting gene expression without changing the sequence. In colorectal cancer, hypermethylation of the *MLH1* gene promoter is associated with microsatellite instability and poor prognosis (Herman, 1998; Wentzensen, 2017).

10.3.2.2 Histone modifications

Histone modifications can influence chromatin structure and gene expression. In cancer, altered histone acetylation patterns can be indicative of disease progression and response to therapies (Baylin & Jones, 2011; Dawson & Kouzarides, 2012).

10.3.2.3 Non-coding RNAs

Non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play roles in regulating gene expression. The miRNA *miR-21* is often upregulated in various cancers and is associated with poor prognosis (Liu, 2015; Tazawa, 2015).

10.3.3 Protein biomarkers

In the realm of healthcare and medical research, the quest to enhance patient outcomes and advance precision medicine remains at the forefront of scientific endeavors. One of the pivotal areas driving this pursuit is the identification and utilization of protein prognostic biomarkers. These biomarkers play a crucial role in revolutionizing diagnosis, prognosis, and treatment strategies, particularly in the context of complex diseases such as cancer, cardiovascular conditions, and neurological disorders.

Professionals and scientists are pivotal players in advancing our understanding of protein prognostic biomarkers and translating their potential into tangible clinical

applications. The chapter serves as a comprehensive exploration of the significance of protein prognostic biomarkers in contemporary healthcare and research, as well as their implications for the future.

Protein prognostic biomarkers, often derived from various sources including blood, tissue, or other biological samples, offer unique insights into disease progression and individual patient responses. Through the identification of specific proteins associated with disease states, researchers and healthcare professionals can gain invaluable information regarding prognosis, therapeutic efficacy, and potential complications. This knowledge forms the cornerstone of personalized medicine, allowing for tailored interventions that optimize patient outcomes and minimize adverse effects.

The study of protein prognostic biomarkers presents a compelling avenue for research and specialization. Understanding the intricate mechanisms underlying these biomarkers and their clinical relevance significantly contributes to the body of scientific knowledge. Furthermore, it lays the foundation for the development of innovative diagnostic tools and targeted therapies, thus shaping the future of healthcare practice.

Healthcare professionals, including clinicians, pathologists, and biomedical scientists, are integral in the translation of protein prognostic biomarker research into clinical practice. By harnessing the potential of these biomarkers, healthcare professionals can refine diagnostic protocols, prognostic assessments, and treatment decisions. Moreover, the integration of biomarker data into patient care facilitates a more personalized and precise approach to addressing complex medical challenges (Catalona et al., 1991; Mottet et al., 2017).

In the realm of scientific research, the quest to unveil novel protein prognostic biomarkers and elucidate their functional roles drives innovation and discovery. By leveraging advanced technologies such as mass spectrometry, genomics, and bioinformatics, scientists can unravel the intricate signatures of protein biomarkers with unprecedented depth and precision. This multidisciplinary approach not only expands our understanding of disease processes but also paves the way for the development of breakthrough therapies and interventions.

Looking ahead, the continued exploration of protein prognostic biomarkers holds immense promise for reshaping the landscape of healthcare. As advancements in technology and research methodologies propel our capabilities to identify and interpret biomarker data, the potential for earlier disease detection, refined prognostic assessments, and personalized treatment strategies becomes increasingly tangible.

Protein prognostic biomarkers stand as a linchpin in the pursuit of precision medicine. By harnessing the collective expertise of professionals, the translation of biomarker data into clinical practice continues to redefine the boundaries of patient care and scientific innovation. The future of healthcare undoubtedly rests on the pivotal role of protein prognostic biomarkers in shaping a more personalized and effective approach to addressing the diverse array of medical challenges that confront us.

Protein biomarkers are molecules expressed in the blood or tissues that provide information about the disease state.

10.3.3.1 Circulating proteins

Proteins in blood or other bodily fluids can indicate disease presence and progression. *PSA* monitors prostate cancer progression and response to therapy (Catalona et al., 1991; Mottet et al., 2017).

10.3.3.2 Tumor antigens

Tumor antigens are proteins expressed by cancer cells but not by normal cells or present at higher levels in cancer cells. The *CA-125* antigen is used to monitor ovarian cancer and assess treatment response (Bast et al., 1983; Moore, 2008).

10.3.3.3 Prognostic protein panels

Prognostic protein panels measure multiple proteins simultaneously to provide a comprehensive prognostic profile. The *Gleason Score* in prostate cancer uses histological patterns to predict tumor behavior and patient prognosis (Epstein, 2005; Mottet, 2020).

10.3.4 Imaging biomarkers

In the realm of healthcare research, imaging prognostic biomarkers play a pivotal role in the identification and prognosis of various diseases. With a target audience including students, healthcare professionals, and scientists, it is imperative to delve into the significance of these biomarkers and their impact on advancing medical knowledge and patient care.

Imaging prognostic biomarkers refer to biological indicators that are identified through imaging techniques and are used to predict the progression or outcome of a disease. These biomarkers can be identified through various imaging modalities such as magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT), and single-photon emission computed tomography. By analyzing the physiological and molecular processes within the body, these imaging biomarkers provide critical information that aids in prognosticating disease progression and treatment response.

The understanding the intricate role of imaging prognostic biomarkers is essential for driving progress in medical research. These students are at the forefront of exploring new frontiers in healthcare, and grasping the nuances of imaging biomarkers equips them to contribute to groundbreaking research in the field. Additionally, healthcare professionals benefit from this knowledge by integrating the latest findings into clinical practice, thereby enhancing patient care and treatment strategies.

In the context of scientific research, imaging prognostic biomarkers serve as indispensable tools for unlocking the mysteries of various diseases. Whether it's

cancer, neurodegenerative disorders, cardiovascular conditions, or other ailments, the ability to accurately predict disease progression and tailor treatments accordingly can have a profound impact on patient outcomes. Moreover, the growing emphasis on personalized medicine underscores the significance of imaging biomarkers in identifying patient-specific prognostic indicators.

One of the key areas where imaging prognostic biomarkers have made significant strides is in oncology. Through advanced imaging techniques, researchers and clinicians can identify specific biomarkers that provide insights into tumor behavior, response to therapy, and overall patient prognosis. This has catalyzed the development of targeted therapies and precision medicine, revolutionizing the landscape of cancer treatment and management.

Furthermore, the integration of AI and machine learning algorithms has further augmented the potential of imaging prognostic biomarkers. These advanced technologies enable the extraction of intricate patterns and features from imaging data, leading to more accurate prognostic assessments and personalized treatment strategies. As a result, the intersection of imaging biomarkers and AI presents a promising avenue for future research and clinical applications.

In conclusion, imaging prognostic biomarkers stand as indispensable assets in the realm of healthcare research. Their impact permeates across various domains, from empowering students and healthcare professionals to steering scientific breakthroughs and enhancing patient care. As technological advancements continue to unfold, the potential of imaging biomarkers in predicting disease outcomes and guiding tailored interventions is poised to shape the future of healthcare in profound ways.

Imaging biomarkers involve the use of imaging techniques to evaluate disease characteristics and progression.

10.3.4.1 *Computed tomography imaging*

CT imaging has revolutionized the way we diagnose and treat various medical conditions. In recent years, there has been a growing interest in utilizing CT imaging as a means to identify prognostic biomarkers in individuals. This has significant implications for students, healthcare and professionals as it opens up new possibilities for personalized medicine and targeted therapies. These biomarkers are often structural or functional characteristics that can be identified through detailed analysis of the CT images. By correlating these biomarkers with clinical outcomes, healthcare professionals and researchers can gain valuable insights into disease progression, treatment response, and overall patient prognosis.

CT imaging offers several advantages when it comes to identifying prognostic biomarkers. Its ability to capture detailed cross-sectional images of the body allows for the visualization of anatomical structures with exceptional clarity. Moreover, advances in imaging technology have paved the way for more sophisticated analyses, such as quantitative imaging techniques, which can extract quantitative data from CT images. These capabilities make CT imaging a powerful tool for

identifying and studying prognostic biomarkers. CT imaging represents a rich area of research with numerous opportunities for exploration. From uncovering new prognostic biomarkers to evaluating the predictive value of existing ones, there is much to be discovered in this field. Furthermore, healthcare professionals can leverage CT imaging to tailor treatment strategies based on the prognostic biomarkers identified in individual patients. This personalized approach to medicine holds great promise for improving patient outcomes and reducing the burden of disease.

While the potential of prognostic biomarkers in CT imaging is undeniable, there are challenges that must be addressed. Standardizing image acquisition protocols, establishing robust analysis methodologies, and ensuring the reliability of findings are critical considerations. Additionally, ethical and privacy concerns related to the handling of sensitive medical imaging data must be carefully navigated. As technology continues to advance, the potential of CT imaging in identifying prognostic biomarkers is expected to expand even further. Integration with artificial intelligence and machine learning algorithms holds the promise of automating the identification and interpretation of biomarkers, thereby accelerating the pace of research and clinical translation. The utilization of CT imaging for identifying prognostic biomarkers represents a frontier in medical research and clinical practice. For master students, healthcare professionals, and scientists, this area offers a wealth of opportunities to contribute to the advancement of personalized medicine and the improvement of patient care. By harnessing the power of CT imaging, we can gain deeper insights into disease prognosis and pave the way for more targeted and effective interventions.

CT imaging is used to visualize anatomical structures and assess disease spread. *CT scans* are employed to stage cancers and monitor responses to therapy, such as in lung cancer (Akin, 2020; Müller, 2007).

10.3.4.2 Magnetic resonance imaging

In the ever-evolving landscape of healthcare, technological advancements continue to shape the way we diagnose and treat illnesses. One such innovation that has revolutionized the field is the use of MRI as a prognostic biomarker. Healthcare professionals and scientists are key stakeholders who can greatly benefit from understanding the implications and applications of this remarkable development.

MRI, a noninvasive imaging technique, has been widely utilized in the diagnosis and monitoring of various medical conditions. However, recent research has demonstrated its potential to serve as a prognostic biomarker, providing valuable insights into disease progression and treatment outcomes. This breakthrough has sparked immense interest and holds significant promise for the future of healthcare.

The integration of MRI as a prognostic biomarker presents numerous advantages for healthcare professionals. By leveraging this technology, clinicians are empowered to make more informed decisions regarding patient care. For instance, in the realm of oncology, MRI can offer early indications of tumor response to

treatment, enabling timely adjustments to therapeutic strategies. Furthermore, the ability to predict disease progression through MRI biomarkers facilitates personalized medicine, ultimately enhancing patient outcomes. In addition to its clinical implications, the use of MRI as a prognostic biomarker is of great interest to the scientific community. Researchers are exploring the potential of MRI to identify and characterize biomarkers associated with specific diseases. By elucidating the underlying mechanisms and manifestations of various conditions, scientists can advance their understanding of disease pathophysiology and explore novel therapeutic targets. This paves the way for the development of more effective treatment modalities and pharmaceutical interventions.

Furthermore, the utilization of MRI biomarkers in research settings has the potential to accelerate the pace of medical innovation. As scientists delve deeper into the nuances of disease pathology, the data derived from MRI can serve as a crucial tool for elucidating disease mechanisms and refining prognostic models. This not only enriches our knowledge base but also has implications for the development of precision medicine tailored to individual patient profiles. Despite the immense promise of MRI as a prognostic biomarker, challenges persist in its widespread implementation. Standardization of imaging protocols, establishment of reference ranges, and integration of MRI biomarkers into clinical practice are areas that require further attention and meticulous research. Moreover, issues related to accessibility and cost-effectiveness must be addressed to ensure equitable distribution of this technology across diverse healthcare settings.

The emergence of MRI as a prognostic biomarker signifies a pivotal juncture in the continuum of healthcare innovation. For healthcare professionals, its incorporation offers enhanced diagnostic and prognostic capabilities, propelling the paradigm toward more personalized and effective patient care. Simultaneously, the scientific community stands to gain invaluable insights into disease mechanisms and therapeutic targets through the utilization of MRI biomarkers. While challenges persist, the potential for transformative impact underscores the importance of continued exploration and advancement in this burgeoning field. As we navigate this frontier, collaboration between healthcare professionals and scientists will be pivotal in unlocking the full potential of MRI as a prognostic biomarker. MRI provides detailed images of soft tissues and is used in various cancers. *MRI* is used for assessing brain tumors, evaluating their extent, and planning treatment (McDonald, 2019).

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CHAPTER

Predictive biomarkers: guiding personalized cancer therapies

11

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11.1 Introduction

In such an environment, forecasting biomarkers is crucial, and tailored care is becoming a more vital strategy in the never-ending quest to increase the therapeutic efficacy of cancer treatments (Vellichirammal et al., 2021). Because so many different genetic profiles may influence how cancer develops, progresses, and responds to treatment, cancer is an extremely complicated disease. Genetic variations among individuals necessitate more complex and customized therapies than the one-size-fits-all nature of traditional therapeutic techniques. Predictive biomarkers, whether genetic or molecular, may show a patient's propensity to react differently to a certain treatment. By examining the specific characteristics of the individual's cancer, these biomarkers help physicians choose the most suitable course of action for medical care, therefore reducing the adverse effects of standard drugs (Sun et al., 2023). Predictive biomarkers show that therapeutic selection techniques are advancing as they migrate away from pharmacological selection and toward a more individualized and tailored approach. Its largest application is as anticipated biomarkers to guide specific medication dosages (Kumar, Pandey, et al., 2024). These medications work by specifically targeting molecular changes in cancerous cells to disrupt the chemical pathways that promote the growth of tumors. Biological indicators such as specific genes or protein expression or abnormalities are known as predictive biomarkers, which help identify the most effective targeted treatments (Jahagirdar et al., 2023). This method eliminates needless exposure to potentially hazardous or inadequate drugs and strengthens the effects of therapy (Angolkar et al., 2022). Furthermore, the introduction of high-throughput technologies like as proteomics and genomics has significantly improved our capacity to detect and evaluate prognostic biomarkers (Pernice et al., 2023).

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11.1.1 The history and development of cancer therapy

Cancer therapy has evolved via an ongoing process involving multiple turning points, starting at a time when there were very few possibilities for treatment and concluding in the present-day era of specialized and targeted therapies. In the past, the most prevalent medical care for malignancy was surgery, which included the removal of tumors (Zhong et al., 2021). Regretfully, this method was not without issues, especially when it comes to treating tumors that metastasized to important organs or illnesses. With the introduction of chemotherapy and radiation therapy in the middle of the 20th century, cancer treatment expanded significantly. While ionizing radiation is used in radiation therapy to precisely target and destroy cancer cells, cytotoxic medications are utilized in therapy to halt quickly multiplying cells from growing (Lazzeroni et al., 2021). By focusing on the precise biochemical changes that caused malignancies, individualized therapies offered a more focused and effective approach than conventional treatments (Ginzac et al., 2018). Drugs that may specifically target molecules associated with cancer and obstruct signaling pathways required for carcinoma survival have recently become accessible due to the identification of monoclonal antigens and small-molecule inhibitors (Afkar et al., 2020). The discovery of the intricate genetic and molecular landscape of forms of cancer has been linked with the growing understanding of tumor biology brought forth by the Human Genomes Project and other genomic projects. Previous findings established that the treatment approach for every patient is determined by the genetic composition of their cancer, therefore paving the way for the discipline of precision medicine. As a result of this paradigm shift, predictively significant biomarkers surfaced, allowing physician to modify patient treatment based on the specific characteristics of each patient's cancer (Hossain et al., 2024).

11.1.2 Increase in personalized care

Customized drugs will soon usher in a paradigm shift in cancer therapy by substituting more focused, targeted techniques for antiquated, all-encompassing ones. To maximize treatment success, an enhanced strategy is needed since every illness is unique and the molecular basis of cancer is growing more complicated (Maroto-Gómez et al., 2023). One major contributing element to the rise of customized healthcare has been the rapid development of genetic profiling technological advances. A plethora of previously unattainable data is now available due to the precise analysis of the genetic and biochemical features of malignancies. With the use of technological advancements like next-generation sequencing (NGS), doctors can now identify unique protein profiles, gene expressions, and anomalies that are unique to every cancer patient (Ophir et al., 2016). These molecular data provide the basis for specific treatment approaches. The foundation of personalized cancer treatment is the creation of unique drug combinations made possible by molecular discoveries (van Schaik, 2008). Targeting chemicals or

processes that are critical to the growth and survival of cancer cells is the aim of these medicines (Mura & Couvreur, 2012).

11.1.3 Predictive biomarkers' function in tailored cancer treatment

A goal of predicting biomarkers in personalized cancer therapy is to assist choose the optimal course of action based on the unique biologic features of each patient's tumor (Özyurt et al., 2023). Physicians may use these biomarkers, which include epidermal growth factor receptor (EGFR), programmed death ligand 1 (PD-L1), and human epidermal growth factor receptor 2 (HER2), to determine the chance of a successful targeted therapy (Susič et al., 2023). Predictive biomarkers lower side effects, boost overall treatment effectiveness, and enhance therapeutic precision. The aforementioned principle facilitates a tailored approach, ensuring that patients get treatments that are most likely to be efficacious against their specific kind of cancer (Moik & Ay, 2022). Predictive biomarkers' function in tailored cancer treatment (see).

11.2 Predictive biomarker principles

Predictive biomarkers are a vital part of personalized therapy since they enable patients to tailor an individual's therapy based on specific features of their cancer. These markers are chemical reaction indicators that provide light on the possibility of a patient experiencing an adverse medication reaction (Hao et al., 2024). They are often of a molecular or genetic origin. The concepts behind predictive biomarkers must be comprehended in order to advance precision medicine's use in cancer therapy. Predictive biomarkers have links to the physiological processes that underlie the emergence and progression of cancer (Machiels et al., 2023). To identify biomarkers linked to a therapy's response, clinicians may use molecular or genetic examination of a cancer. Using this knowledge might reduce adverse effects, increase the efficacy of therapy, and get rid of potentially dangerous or unnecessary drugs (Wagner et al., 2023).

11.2.1 The biological basis of biomarkers

Biomarkers are indicators of a disease's development, course, and response to therapy that are found in many illnesses, including cancer. These tools are based on the intricate interplay between molecular, genetic, and epigenetic modifications inside cells (Ben Moussa et al., 2024). Genetic abnormalities, changes in protein concentrations, or changed gene expressions that fundamentally reflect the molecular properties of tumors may all be considered biomarkers, especially when it comes to cancer (Li et al., 2023). Genetic markers, such as differences in DNA sequence, make up the majority of the molecular foundation. Gene

abnormalities such as mutations, amplifications, and deletions are responsible for the development and spread of cancer. Gene alterations affecting EGFR, Kirsten rat sarcoma viral oncogene homolog (KRAS), or BRAF, for example, might change to cancer cells behave and react to different treatments. Significant effects are also attributed to epigenetic alterations such as DNA methylation and histone acetylation (Ritterhouse & Gogakos, 2022). These modifications provide command over the function of genes without altering the core DNA sequence. An oncogene activation or tumor suppressor gene silencing is frequently linked to atypical epigenetic mutations in cancer (Teng et al., 2023).

11.2.2 The relevance of genetic mutations

Genetic mutations are changes in the DNA sequence that can significantly affect a cell's capacity to function and are connected to the emergence of a number of illnesses, including cancer (Wen et al., 2024). The two main categories into which genetic changes connected to cancer can be separated are mutations affecting drivers and passenger alterations (Al-Toubat et al., 2023). Driver mutations provide affected cells a selective growth advantage, which plays a direct role in the initiation and progression of cancer. These mutations usually include oncogenes or genes that, when changed, promote uncontrolled cell growth and division. Examples include alterations in the EGFR gene in lung cancer or the BRAF gene in melanoma. Finding these mutations may aid in understanding the mutational landscape of a particular type of cancer, even though they may not be the main cause of carcinogenesis. Beyond just being the cause of cancers, genetic abnormalities are significant for other reasons. Furthermore, mutations impact a patient's response to therapy (Pezzuto et al., 2023). For instance, certain mutations may make cancer cells more vulnerable to focused therapies. However, mutations in certain genes, such as those linked to DNA repair mechanisms like BRCA1 or BRCA2, may cause resistance to a given medication (see).

11.2.3 Tumor microenvironment and biomarker dynamics

Tumor microenvironment (TME) is a complex ecosystem consisting of several cell types, extracellular matrix components, and signaling molecules that surround a tumor (Haratani et al., 2023). Understanding the interaction between the TME and biomarker dynamics is crucial for comprehending the onset, progression, and therapeutic response of cancer. Numerous biomarkers, including genetic, immunologic, and proteomic components, are present in the TME. Genetic alterations in both the malignant cells and the encircled stromal cells affect the TME's complexity (He et al., 2023). The presence of TME dynamics is also affected by proteomic modifications including changed protein expression in cell signaling and the immune system. Immune-related markers that affect the way the tumor interacts with the body's immune system are crucial in the TME. Immunology biomarkers

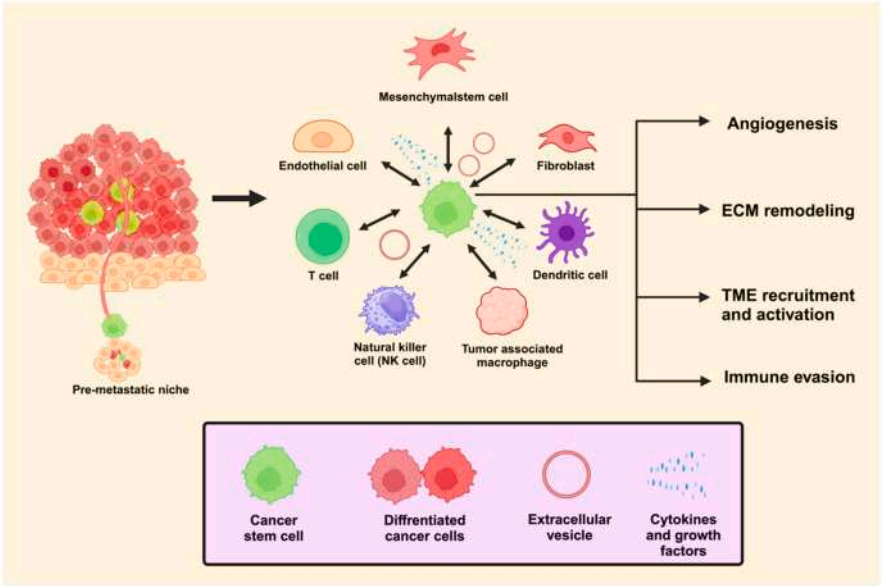

FIGURE 11.1

The relationship between cancer stem cells (CSC) and the surroundings of the cancer. CSCs are the main factor in the development and metastasis of tumors. One of the main factors influencing the development of cancer and resistance to medication treatments is the interaction between CSCs and the tumor microenvironment, which is made up of T cells, natural killer cells, dendritic cells, tumor-associated macrophages, mesenchymal stem cells, fibroblasts, and endothelial cells. These cells interact with CSCs to promote cancer growth and immune evasion by the production of growth factors, cytokines, and extracellular vehicles (no date).

include the expression of programmed cell death ligand 1 (PD-L1) on cancer cells. The higher levels of PD-L1 suggest a possible immune check point inhibitor response, highlighting the importance of TME biomarkers in predicting treatment outcomes (Kim et al., 2024). TME-associated biomarkers may fluctuate throughout treatment, impacting both therapeutic response and resistance. To adapt treatment strategies in response to these changes, it is essential to continually monitor the dynamics of biomarkers inside the TME (see Fig. 11.1) (Tufail, 2023).

11.3 Technological advancements

Technology has revolutionized the area of cancer explore and therapy, opening the door to more precise and customized treatment plans. High-throughput sequencing methods, such as NGS, enable comprehensive genomic profiling to identify genetic

changes. The single-cell study illustrates the intratumor heterogeneity, as do advanced diagnostic imaging techniques like MRI and PET (Chandnani et al., 2023). CRISPR technology makes gene editing feasible for explore and potential cures. Artificial intelligence (AI) facilitates data analysis and pattern recognition. All of these advancements are driving oncology to deliver more effective, targeted, and personalized cancer therapy, offering patients new hope for improved outcomes (Sharma et al., 2023).

11.3.1 Next-generation sequencing techniques

NGS methods are a major development in genomics revolutionizing our understanding of cancer biology and enabling personalized therapy (Wang et al., 2016). NGS is able to sequence DNA or RNA quickly and accurately, enabling previously unheard-of levels of speed and precision in the identification of genetic alterations (Boström et al., 2017). The bulk of mutations linked to disease is located in these areas, even though they comprise a very tiny portion of the whole genome (Xia et al., 2023). The primary objective of whole exome sequencing (WES) is this. WES is one of the most widely used NGS technologies in cancer exploration and diagnosis due to its reduced cost and capacity to collect genetic data that is clinically useful. Enhancing DNA-based explore, RNA sequencing, or RNA-Seq, yields information on patterns of gene expression. It improves our knowledge of genes that govern health and disease through the identification and measurement of RNA molecules. RNA-Seq is useful in identifying expression abnormalities, alternative splicing events, and fusion genes that may stimulate the development of cancer (Gindin & Hsiao, 2022).

11.3.1.1 Liquid biopsy: revolutionizing biomarker identification

One of the primary applications of liquid biopsy is the identification of circulating tumor DNA (ctDNA), which is composed of minuscule DNA fragments released into the bloodstream by cancer cells (Aquino & Pascut, 2024). ctDNA analysis may be used to detect genetic mutations, copy number variations, and other tumor-specific genomic alterations (Licata et al., 2023). With this information, therapeutic decisions may be made; understanding the molecular landscape of the cancer is crucial. Liquid biopsy is especially useful when obtaining tissue samples is challenging or impracticable, as is the case with metastatic cancers or tumors located in physically challenging-to-reach areas (Raez et al., 2023).

11.3.1.2 Technology incorporation into clinical practice

The practical application of cutting-edge technologies like liquid biopsies and NGS has dramatically altered the way that cancer care is provided (Ashique et al., 2024). These technologies provide doctors access to extensive genetic data, allowing them to create individualized treatment plans depending on the particulars of each patient's cancer (Winkelmann et al., 2017). AI facilitates data analysis, pattern detection, and treatment response prediction. In the era of precision oncology,

therapeutic efficacy, and diagnostic accuracy are increased by the smooth integration of these technologies into routine clinical processes, which ultimately improves patient outcomes (Charron et al., 2022).

11.4 Dynamic nature of cancer and adaptive treatment strategies

Cancer is a dynamic illness characterized by genetic growth, clonal variation, and adaptability. Consequently, therapeutic approaches must also be flexible to effectively combat the disease's ever-changing nature. Cancer cells are constantly undergoing genetic changes, which result in subclones with distinct molecular profiles (Strobl et al., 2023). This intratumor heterogeneity hinders the development of medications that can target the many cell types inside a tumor. Adaptive treatment methods consider the dynamic character of cancer and tailor medications in response to real-time assessments of the tumor's genetic and molecular composition (Yang et al., 2023). Liquid biopsies and NGS allow clinicians to adapt treatment plans to dynamic changes in the tumor's genetic composition. An essential part of adaptive strategies is the identification of resistance mechanisms. Because cancer cells have adapted to the selection pressures imposed by previously effective treatments, they may develop resistance to such therapies (Bayer et al., 2022). These resistance pathways can be better understood by ongoing molecular profiling, which enables prompt modification of treatment tactics to overcome or avoid resistance and enhance therapeutic outcomes. Immunotherapy is a novel approach to cancer treatment that exhibits adaptability in the face of tumor behavior that is always shifting. Immunotherapy often has long-lasting benefits and uses the immune system to target cancer cells. Immunological escape pathways, however, may arise from tumor heterogeneity and evolution (Jain et al., 2023). Personalized cancer vaccines and the combination of checkpoint inhibitors are two adaptive immunotherapy strategies that allow for therapeutic modification depending on immune responses. Another illustration of the dynamic approach to cancer therapy is adaptive clinical trials. These trials allow alterations based on data collected throughout the study, increasing the possibility of effective therapies and speeding the application of explored findings in clinical practice (Johnson et al., 2024). AI enhances adaptive approaches' ability to assess vast, complex information, identify patterns, and predict treatment outcomes. AI technologies can help doctors make decisions in real time by assisting them in selecting the most effective courses of action based on the tumor's dynamic genomic landscape (Corredor et al., 2023).

11.4.1 Understanding cancer as a dynamic entity

Cancer's genetic changes, clonal development, and adaptability are what make it dynamic. Tumor cells have diverse molecular profiles and diverse populations as a

result of their continuous evolution (Hermann & Sainz, 2018). Its dynamic nature makes the development of static, one-size-fits-all remedies challenging. The recognition of cancer as a dynamic organism emphasizes the need for adaptive tactics, wherein therapies are tailored based on real-time assessments of the evolving genetic and molecular landscape. Comprehending the ever-changing dynamic characteristics of cancer is crucial for developing customized, precise treatments that can adapt to the disease's ever-changing properties, ultimately improving treatment efficacy and patient outcomes (Wu et al., 2024).

11.4.1.1 *Biomarker-guided adaptive treatment approaches*

Biomarker-guided adaptive treatment approaches, which tailor treatments based on real-time molecular information and acknowledge the dynamic nature of tumors, constitute a unique paradigm in cancer therapy (Wolf et al., 2022). The identification of predictive biomarkers allows clinicians to monitor treatment outcomes and adjust strategies in response to genetic alterations in tumors. Continuous biomarker analysis maximizes the efficacy of treatments by allowing timely adjustments to treatment plans as the cancer develops resistance or adapts. Immunotherapy response prediction, particularly when using immune checkpoint inhibitors, is mostly reliant on biomarkers like PD-L1 expression. Through monitoring these markers throughout treatment, adaptive strategies can provide modifications or a combination of therapies that enhance the immune system's capacity to fight tumors (Bamodu et al., 2023). Liquid biopsy is a noninvasive method of detecting circulating tumor DNA that allows real-time monitoring of genetic changes in response to treatment. This continuous evaluation facilitates the identification of novel resistance mechanisms and the direction of adaptive adjustments to maintain therapeutic efficacy. Biomarker-guided adaptive approaches are highly effective in clinical studies. These trials employ molecular profiling to classify patients based on specific biomarkers, allowing treatment arms to be modified adaptively in response to interim results (Barker et al., 2023). This tactic accelerates the discovery of effective treatments and improves trial efficiency. AI plays a critical role in biomarker explore because of its ability to swiftly analyze massive information and identify trends that inform adaptive strategies. Considering that machine learning algorithms can predict treatment responses and help with adaptive therapy procedures can be more accurate (Adamaki & Zoumpourlis, 2021).

11.4.1.1.1 Overcoming challenges in adaptive therapies

While adaptive therapies are very promising for the treatment of cancer, several challenges must be addressed before their full potential can be realized. A significant challenge is locating trustworthy biomarkers that have undergone clinical verification (Singh et al., 2024). The dynamic nature of cancers and intratumor heterogeneity pose difficulties in identifying reliable markers that will guide individualized treatment plans. For the biomarker assessments to be accurate and repeatable across different platforms and labs, standardized techniques are

needed. Another challenge is coming up with rapid and effective ways to discover newly emerging resistance mechanisms (Arif et al., 2023). Fast tumor development can lead to genetic alterations that render previously effective therapies ineffective. Continuous monitoring using techniques such as liquid biopsy is essential, but it is logistically and analytically challenging to swiftly analyze and act upon this dynamic data. For adaptive treatments to be effective, clinical trial design is essential. Due to their frequently rigid and predetermined nature, standard trial structures may not be the best match for adaptive approaches (Din et al., 2023). Despite administrative and legal challenges, adaptive trial designs must be used to rapidly identify effective treatments and advance them toward clinical application. Moreover, integrating AI into healthcare decision-making raises issues with data protection, standardization, and interpretability. It is necessary to address any biases in algorithms and ensure the ethical use of AI to foster faith in these technologies (Cai et al., 2023).

11.5 Collaboration in advancing biomarker explore

Explorer, physician, industry partner, and regulatory body collaboration is essential to the progress of biomarker explore (Wagner & Srivastava, 2023). This methodology expedites the development and implementation of novel biomarkers in clinical settings. Interprofessional collaboration between basic scientists and clinicians is necessary to convert laboratory results into clinically relevant biomarkers. Clinicians provide crucial insights into the practical challenges involved with biomarker adoption, ensuring that explore aligns with real clinical needs. Working together with the industry is crucial to turning potential biomarkers into therapies and diagnostic tools. Partnerships with biotechnology and pharmaceutical companies assist in the development of tests, instruments, and tailored medications needed for biomarker-driven precision medicine (Achi et al., 2024).

11.5.1 Interdisciplinary approaches in biomarker discovery

The confluence of diverse information tremendously improves the dynamic field of biomarker development, increasing the chances of finding robust and clinically effective biomarkers. This is the point at which interdisciplinary methods must be used. Scientists can better traverse the complexity of biological systems by integrating different areas (Xie et al., 2021). Collaboration between physicians and fundamental biomedical scientists is essential for both biological and therapeutic reasons. Although biologists could concentrate on comprehending the molecular subtleties of illnesses, clinicians offer vital perspectives on the realistic elements of patient treatment. This multidisciplinary partnership guarantees that biomarkers have biological relevance as well as convenience and performance in medical applications (Kapoor et al., 2023). When combined, the enormous volumes

generated by high-throughput technologies might be effectively handled by experts in genomics and bioinformatics. Genome experts help identify the genetic bases of many diseases, whereas bioinformaticians provide methods for processing, analyzing, and interpreting data. This sort of collaboration is necessary to identify patterns, correlations, and potential biomarkers in complex genomic data. Technology and engineering integration play an integral part in the advancement of novel methods for biomarker analysis and detection (Bamodu et al., 2023). Biosensors, imaging technologies, and microfluidics developed by engineers can provide sensitive and accurate tools for biomarker detection. By using these technologies in biomarker studies, biomarker detection accuracy and efficiency are increased. Immunology and biomarker development are key to comprehending the immune response to disease (Matharoo-Ball et al., 2007).

11.5.1.1 *Industry–university cooperation*

Academic contributions to fundamental explore are beneficial because they often push the boundaries of scientific understanding and explore new avenues for biomarker discovery (Xiao & Liu, 2019). Industry partners provide the infrastructure, technological resources needed for large-scale validation, comprehensive clinical trials, and commercialization. This collaborative synergy enhances the translational potential of biomarker results by bridging the gap between theoretical explore and practical clinical applications. Industry partners can provide financial support, access to state-of-the-art technology, specialist manufacturing and regulatory affairs expertise, and potential biomarkers to transform them into diagnostic tools and therapeutic therapies (Lacombe et al., 2013).

11.5.1.1.1 Pharmaceutical companies' role in biomarker-driven therapies

Pharmaceutical companies are important contributors to the development of biomarker-driven medications because they offer the resources and expertise required to transform precision medicine from a theoretical therapy to a workable solution. Biomarkers are used by pharmaceutical companies to find and assess therapeutic targets during drug development and target identification procedures (Kim & Prasad, 2021). Biomarkers contribute to the advancement of drug development strategies by enabling the identification of patient populations most likely to benefit from a particular medicine. This targeted approach reduces expenses and development time while increasing the effectiveness of medications (Fariha et al., 2022). Companion diagnostics-focused pharmaceutical companies invest in the development of these tests, which identify specific biomarkers to guide the administration of a corresponding medicine. These diagnostics offer a more customized and effective approach by identifying patients who are most likely to benefit from a certain treatment. To improve patient categorization accuracy and increase the likelihood of demonstrating treatment efficacy, biomarkers are incorporated into clinical trial designs. Identifying subpopulations that respond favorably to a certain medicine is made simpler using this strategy, which facilitates

market access and regulatory approvals. Real-world evidence creation after marketing pharmaceutical companies track the long-term safety and effectiveness of biomarker-driven medications, which contributes to the production of real-world data (Sufyan et al., 2023). This ongoing assessment improves therapy suggestions and provides information for clinical practice. Explore institutions and partnerships with academics and explore groups to promote innovation in biomarker development. Working with outside experts can provide pharmaceutical companies access to state-of-the-art explore and other perspectives, which can speed up the discovery of relevant biomarkers (Liang et al., 2022).

11.6 Case studies and clinical trials

11.6.1 Successful applications of biomarker-driven therapies

Biomarker-driven medicines have shown remarkable promise in several medical domains, transforming patient care by personalizing treatments based on individual characteristics. Biomarkers guide the use of targeted medications in oncology (Machiels et al., 2023). For example, patients with HER2-positive breast cancer benefit from Herceptin therapy, which directly targets the HER2 protein. In a similar vein, specific medicines like vemurafenib have been developed in response to the identification of certain genetic abnormalities, such as the BRAF mutations in melanoma. Immunotherapy biomarkers, namely PD-L1 expression, are critical in predicting the immune system's response to immune checkpoint inhibitors like pembrolizumab and nivolumab (Redman et al. 2020). Impressive results have been shown in the therapy of lung cancer, melanoma, and renal cell carcinoma, among other cancers. Utilizing biomarkers for infectious diseases allows for some degree of customization in the treatment of infectious disorders. As an example, the HIV viral load is a biomarker that is used to monitor antiretroviral medication effectiveness and guide treatment adjustments to optimize outcomes (Zhao et al., 2022). Biomarkers like troponin are used in the diagnosis and treatment of cardiovascular disease. Elevated troponin levels indicate myocardial damage and guide the appropriate course of therapy. Neurological disease biomarkers aid in both diagnosis and therapy. For instance, tau and beta-amyloid proteins are biomarkers for Alzheimer disease that help in early detection and tracking the illness's progression (see Fig. 11.2) (Campuzano et al., 2023).

11.6.2 Lessons learned from clinical trials

Clinical trial outcomes have yielded valuable insights that facilitate the development of novel medicines and the ongoing progress of medical explore (Friedlander et al., 2016). Numerous significant conclusions have come from these investigations, including different patient groups that are valuable through clinical explore

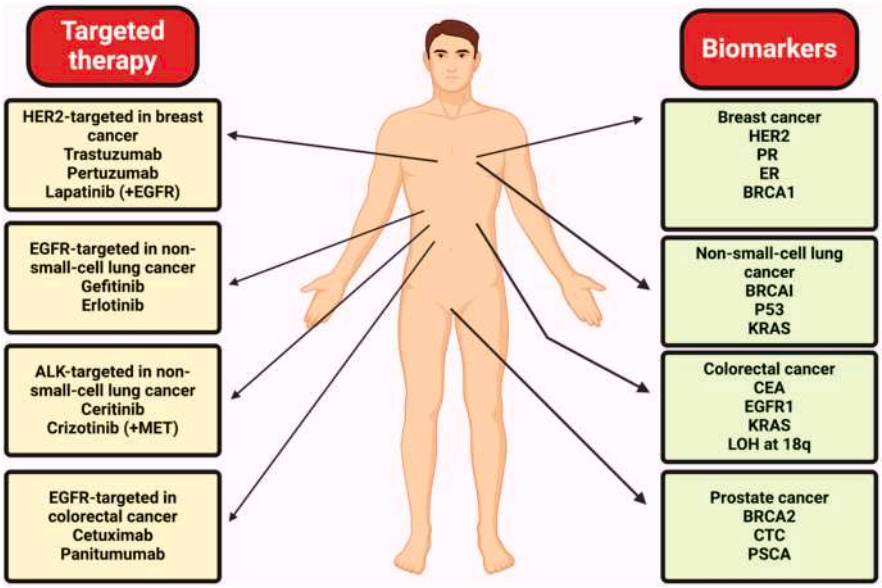

FIGURE 11.2

Personalized medications and biomarkers for different types of cancer.

(Sharma et al., 2024). Including people with different demographic origins ensures that therapy responses are representative of the greater patient population, which advances the development of inclusive and equitable therapies (Kumar, Sharma, et al., 2024). This is emphasized by the fact that the development of biomarker-driven therapies requires robust biomarker validation (Ashique et al., 2024). Beyond their association with clinical outcomes, biomarkers also need to be able to predict treatment responses with any degree of accuracy (Knapen et al., 2020). Errors in the biomarker validation process may lead to inaccurate predictions and worse than optimal patient outcomes. Standard, fixed-design clinical investigations are being supplemented by adaptive trial designs. These allow for modifications at any point during the study process based on preliminary results, increasing effectiveness and accelerating the identification of effective treatments (Pathak et al., 2024). Patient-centric approaches have emphasized patient-centricity more to recognize the importance of patient-reported outcomes and experiences (Cech & Oberlies, 2023). Patients' perspectives enhance the relevance and application of medicines when they are considered in trial design and outcome monitoring. The acknowledgment of cooperative endeavors and data-sharing initiatives as essential components of successful clinical explore has been increasing. The process of discovery is accelerated by shared data since it allows for cross-study validation of findings. Strict regulatory management of clinical trials emphasizes the necessity of stringent regulatory supervision to ensure patient safety and the reliability of

study findings. Strict moral and legal requirements protect explore participants and maintain the study's validity (Wills et al., 2023).

11.6.3 Impact on patient outcomes

Clinical trials have a profound impact on patient outcomes, revolutionizing the field of medicine and considerably improving the quality of life for patients suffering from a variety of ailments (Fulton-Ward & Middleton, 2023). This evolution ensures that patients receive the most effective and scientifically validated therapy by taking into account the most recent advancements in medical explore (Pirzadeh et al., 2024). Extended survival and enhanced quality of life: Explore has shown that certain treatments can increase patient survival rates and enhance their quality of life (Harkin et al., 2023). The outcomes of well-conducted clinical trials, which may include cancer treatments or therapies for chronic conditions, have a direct and lasting influence on the well-being of patients. Clinical studies validate the efficacy of new diagnostic tools, allowing for earlier and more accurate detection of sickness. This early discovery allows for prompt therapy, which eventually enhances patient outcomes (Alnajar et al., 2023).

11.7 Future perspective

The use of predictive biomarkers to guide personalized cancer therapies appears to have a promising future. It is expected that as technology develops, finding new biomarkers will become more difficult, which will lead to a deeper understanding of the intricate molecular landscape of cancer (Azari et al., 2024). Proteomics, metabolomics, and genome developments are examples of omics technology advancements that will facilitate the development of targeted therapies and help identify new predictive biomarkers (Venkatesalu et al., 2024). The use of AI and machine learning in biomarker explore will enhance the analysis of complex data sets by spotting minute patterns and interactions that can escape conventional probes (Davey & Miller, 2024). This ingenious tactic might lead to the identification of previously unidentified biomarkers and the development of more precise prediction models (Liang et al., 2024). In addition, multi-omics approaches will likely become more common in the future due to the interaction of several biological levels. Integrating transcriptomic, proteomic, epigenomic, and genomic data will provide a comprehensive understanding of cancer biology (Zhou et al., 2024). As a result, more accurate prediction biomarkers reflecting the intricacy of individual cancers will be produced. Real-time biomarker dynamics monitoring by liquid biopsy and other noninvasive techniques is becoming more and more significant as the field advances (Mali & Dahivelkar, 2024). This dynamic review will enable doctors to adapt treatment strategies to evolving biomarker profiles, ensuring a more adaptable and personalized approach to cancer care. In the future,

predictive biomarkers may change cancer treatment by enabling the development of personalized medications that enhance patient outcomes and quality of life (Chen et al., 2024).

11.8 Conclusion

In conclusion, predictive biomarkers are ushering in a new era of precision medicine by revolutionizing the delivery of individualized cancer therapies. The journey from the discovery of early indicators, such as HER2 in breast cancer, to the current landscape of many genetic and molecular markers, demonstrates the enormous progress in understanding the complexity of cancer. These markers are critical not just for diagnosis but also for tailoring care regimens, optimizing drug efficacy, and mitigating adverse effects. The future of predictive biomarkers is generating excitement because of technological advancements and an expanding knowledge of cancer biology. Technological developments in AI, multi-omics, and liquid biopsy real-time monitoring are creating a more dynamic, all-encompassing, and personalized cancer care environment. Partnership between academia, business, and healthcare stakeholders is critical to translating biomarker discoveries into

Table 11.1 Predictive biomarkers' function in tailored cancer treatment.

Biomarker	Cancer type	Targeted therapy	References
HER2	Breast cancer	Trastuzumab (Herceptin)	Kirkham et al. (2023)
EGFR	Nonsmall cell lung cancer	Erlotinib, Gefitinib	Hussain et al. (2022)
BRAF V600E	Melanoma	Vemurafenib, Dabrafenib	Kirkham et al. (2023)
KRAS (G12C)	Colorectal cancer	Sotorasib	Moreta-Moraleda et al. (2023)
ALK	Nonsmall cell lung cancer	Crizotinib, Alectinib, Ceritinib	Ladaycia et al. (2021)
PD-L1	Various	Pembrolizumab, Atezolizumab	Ladaycia et al. (2021)
BRCA1/ BRCA2	Ovarian, Breast, Prostate	Olaparib, Rucaparib, Niraparib	Kirkham et al. (2023)
KIT	Gastrointestinal stromal tumors	Imatinib, Sunitinib	Ladaycia et al. (2021)
EML4-ALK	Nonsmall cell lung cancer	Crizotinib, Alectinib, Ceritinib	Hussain et al. (2022)
ROS1	Nonsmall cell lung cancer	Crizotinib, Entrectinib	Hussain et al. (2022)

Table 11.2 Different genetic mutations and their significance in cancer.

Genetic mutation	Significance in cancer	Examples	References
EGFR	Oncogene promotes cell growth and survival	Nonsmall cell lung cancer	Pezzuto et al. (2023)
BRAF	Oncogene regulates cell division and differentiation	Melanoma, colorectal cancer	Dcruz et al. (2023)
KRAS	Oncogenes involved in signaling pathways	Colorectal cancer, pancreatic cancer	Di Giorgio et al. (2023)
TP53	Tumor suppressor regulates the cell cycle and apoptosis	Various cancers, including breast, lung, and colorectal	Sun et al. (2022)
HER2	Oncogene promotes cell growth	Breast cancer, gastric cancer	Valenza et al. (2024)
BRCA1/ BRCA2	Tumor suppressor involved in DNA repair	Breast, ovarian, and prostate cancers	Sørensen (2018)
ALK	Oncogenes involved in cell growth and division	Nonsmall cell lung cancer	Toyokawa et al. (2023)
PIK3CA	Oncogene regulates cell proliferation and survival	Breast, colorectal, and ovarian cancers	Cai et al. (2020)
PTEN	Tumor suppressor regulates cell growth and division	Endometrial, prostate, and breast cancers	Chai et al. (2022)
JAK2	Oncogenes involved in signaling pathways	Myeloproliferative neoplasms (e.g., Polycythemia vera)	Glück et al. (2022)

clinically meaningful treatments. Customized treatment plans have the potential to revolutionize the standard of care as predictive biomarkers influence the development of targeted medications and immunotherapies. Ultimately, the use of predictive biomarkers in clinical practice improves patient outcomes, provides clinicians with more information to aid in decision-making, and advances the objective of creating personalized cancer treatments that account for the unique circumstances of each patient (Tables 11.1 and 11.2).

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CHAPTER

Monitoring treatment response with cancer biomarkers

12

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12.1 Introduction

The discovery of blood glucose as a biomarker for diabetes in the early 20th century marked a pivotal moment in the history of medical diagnostics. This breakthrough demonstrated how specific biological substances could be measured to detect and monitor diseases, laying the foundation for the modern field of biomarkers (Krentz & Hompesch, 2016). Since then, biomarkers have become indispensable tools in healthcare, providing critical insights into the diagnosis, prognosis, and treatment and therapeutic response monitoring of a wide range of conditions (Frank & Hargreaves, 2003). With advances in molecular biology, genetics, and biotechnology, the scope of biomarkers has expanded dramatically. Today, biomarkers encompass a vast array of biological molecules—including DNA, RNA, proteins, and metabolites—that can reflect the underlying mechanisms of diseases. They have transformed medicine's landscape, enabling early disease detection, predicting therapeutic responses, and guiding personalized treatment strategies (Bodaghi et al., 2023).

Cancer biomarkers are measurable biological molecules found in blood, other body fluids, or tissues that indicate the presence of cancer or provide information about the cancer's behavior, progression, or response to treatment (Das et al., 2024). These biomarkers can be genes, proteins, metabolites, or other molecular alterations that are specifically associated with cancer cells. Cancer biomarkers serve as key indicators that help in various aspects of cancer management, including early detection, diagnosis, prognosis, therapeutic response prediction, and treatment efficacy monitoring (Zhou et al., 2024).

12.2 How cancer biomarkers reflect treatment response

Various molecular changes in normal cells can lead to cancerous cells. When those molecular changes can be used to check the prognosis or state of cancer progression, they are then termed as biomarkers for that disease (Sarhadi & Armengol, 2022). In cancer cells, genetic mutations, amplifications or translocation of oncogenes or tumor suppressor genes act as prime biomarkers for cancer, such as, KRAS, BRAF, TP53 (Dakal et al., 2024;

The Potential of Cancer Biomarkers. DOI: https://doi.org/10.1016/B978-0-443-29279-8.00012-0

Sinkala, 2023). Epigenetic changes, like DNA methylation and histone modification, can alter gene expression without actually changing the DNA sequence, contributing to cancer progression and serving as biomarkers, such as, hypermethylation of the MGMT gene (O6-methylguanine-DNA methyltransferase, a gene repair enzyme) in glioblastoma (brain tumor) (Gibney & Nolan, 2010; Lu et al., 2020). Similarly, the presence of cancer cells in the body can trigger immune response of the body to produce antigen, or the cancerous cells can themselves produce proteins or metabolic products that can be marked as biological markers for specific cancer, such as, CA-125 in ovarian cancer or elevated lactate dehydrogenase in lymphoma (Gonzalez et al., 2018; Pardoll, 2015).

Cancer biomarkers provide valuable insights into treatment response by reflecting changes in the disease's biology. Stable biomarker levels suggest that the disease is in a stable state, though it doesn't confirm treatment's success. It only reflects that cancer is not worsening, but does not provide information about whether the tumor is shrinking or being effectively controlled (Kaushal et al., 2022). An increase in biomarker levels may signal that the cancer is progressing or the treatment is not working. Monitoring biomarkers can also reveal if the cancer has developed resistance to the current treatment, prompting adjustments to the therapeutic approach. Depending upon result of biomarker production, there are correlated with their increasing or decreasing level to assess cancer progression (Passaro et al., 2024).

12.3 Types of cancer biomarkers used in monitoring treatment response

In the monitoring of drug response during cancer treatment, employing multiple biomarkers significantly enhances the precision and reliability of assessments. This strategy utilizes the unique strengths of each biomarker, offering a more comprehensive evaluation of treatment efficacy (Ben-Hamo et al., 2020; Kovács et al., 2023). For instance, one biomarker may specifically quantify tumor burden, while another may reveal the impact on a specific molecular pathway targeted by the therapy. Additionally, certain biomarkers may be more sensitive to early changes in treatment response, whereas others are more indicative of long-term outcomes. By integrating various biomarkers, clinicians gain a nuanced understanding of the tumor's reaction to therapy, enabling earlier detection of resistance and more effective modification of treatment plans (Sha et al., 2020). This multifaceted approach facilitates a more personalized treatment strategy, optimizing therapeutic outcomes. Table 12.1 enlists various types of biomarkers based upon their molecular composition.

12.4 Technologies and techniques for biomarker detection

Advanced technologies have revolutionized the detection and quantification of cancer biomarkers. Liquid biopsy, tissue biopsy and immunohistochemistry, next-generation

Table 12.1 Classification of biomarkers based upon their molecular composition.

Type of biomarker	Molecular composition	Examples	Specific cancers	References
Protein biomarkers	Proteins/ Enzymes/ Receptors	CA-125, PSA, CEA, HER2/neu	CA-125: Ovarian cancer; PSA: Prostate cancer; CEA: Colorectal cancer; HER2/neu: Breast cancer Alpha-Fetoprotein (AFP): liver cancer, testicular cancer. Human Epididymis Protein 4 (HE4): Ovarian cancer Lactate Dehydrogenase (LDH): Lymphoma, melanoma, testicular cancer HER2 (Human Epidermal Growth Factor Receptor 2): Breast cancer, gastric cancer Thyroglobulin: Thyroid cancer CA 19-9: Pancreatic cancer, gall bladder cancer. NSE (Neuron-Specific Enolase): Small cell lung cancer, neuroendocrine tumors CA 15-3 / CA 27–29: Breast cancer	Claps et al. (2022); Desai and Guddati (2023); Iqbal and Iqbal (2014); Lu et al. (2012); Luo et al. (2021); Tian et al. (2020)
Genetic biomarkers	DNA/RNA mutations	EGFR mutations, KRAS mutations, BRCA1/2	EGFR: Non-small cell lung cancer; KRAS: Colorectal cancer and Lung Cancer; BRCA1/2: Breast and ovarian cancers	Huang et al. (2021); Pecci et al. (2022); Pourmasoumi et al. (2024)
Epigenetic biomarkers	DNA mMethylation	MGMT promoter methylation	MGMT: Glioblastoma; Other cancers with methylation changes affecting treatment response	Butler et al. (2020); Do et al. (2022)
Metabolic biomarkers	Metabolites	2-HG (2-Hhydroxyglutarate)	2-HG: Gliomas with IDH (isocitrate dehydrogenase) mutations	Rudà et al. (2024)
Circulating tumor DNA/ RNA	DNA/RNA/ gene	ctDNA (circulating tumor DNA)	Lung cancer (EGFR mutations), colorectal cancer (KRAS mutations), melanoma and colorectal cancer (BRAF mutations) BCR-ABL Fusion Gene: Chronic myeloid leukemia (CML)	Bethune et al. (2010); Cicenas et al. (2017)
Circulating tumor cells	Cells	CTCs (circulating tumor cells)	Breast cancer, prostate cancer, colorectal cancer.	Lawrence et al. (2023)

sequencing (NGS), mass spectrometry, and proteomics are some of the methods used for the same immunoassays, such as ELISA, use enzyme-linked antibodies for sensitive and specific biomarker detection. Molecular techniques like PCR and qPCR amplify and quantify DNA/RNA, while NGS provides comprehensive genetic information. Mass spectrometry methods like LC-MS offer high sensitivity for proteomics and metabolomics. Microarray technology includes DNA and protein microarrays for gene expression profiling. Biosensors detect biomarkers through changes in electrical signals or light, providing real-time analysis. Nanotechnology-based techniques use nanoparticles and nanosensors for enhanced sensitivity and specificity. Emerging CRISPR-based detection offers highly specific nucleic acid detection, while label-free detection techniques like surface plasmon resonance offer real-time analysis without the need for labels (Nimse et al., 2016).

12.5 Clinical applications of biomarkers in monitoring treatment response

Biomarkers help oncologists select the most effective treatment based on the molecular profile of the tumor, such as using BRAF inhibitors in melanoma with BRAF mutations. These can be used to assess whether a patient is responding to therapy, enabling timely changes to the treatment regimen if necessary. Elevated levels of specific biomarkers posttreatment can signal disease recurrence, prompting further investigation or intervention.

Biomarkers play a critical role in the monitoring and management of various cancers by providing personalized insights into treatment effectiveness and disease progression. For example, CA-125 is used in ovarian cancer to monitor treatment efficacy and detect disease recurrence, with elevated levels often indicating poor response. In prostate cancer, prostate-specific antigen levels are assessed to gauge treatment success, where a decrease suggests effective therapy. Human Epidermal Growth Factor Receptor 2 (HER2)/neu overexpression in breast cancer patients is essential for determining the suitability for HER2-targeted therapies, while Epidermal Growth Factor Receptor (EGFR) mutations in non-small cell lung cancer guide targeted therapy decisions and help monitor resistance. In chronic myeloid leukemia, the presence of Breakpoint Cluster Region-Abelson (BCR-ABL) transcripts is crucial for evaluating response to tyrosine kinase inhibitors. Vascular Endothelial Growth factor (VEGF) levels across various cancers are used to assess the efficacy of anti-angiogenic therapies, where a decrease often signals effective treatment. Alpha-Fetoprotein (AFP) is monitored in liver cancer and germ cell tumors to detect disease progression, and circulating tumor DNA (ctDNA) offers real-time monitoring of tumor dynamics across multiple cancer types, with changes in ctDNA levels reflecting treatment response or emerging resistance. These biomarkers are indispensable tools in modern oncology, allowing for more targeted and effective cancer management. Table 12.2 enlist the cancers with multiple biomarkers for measuring treatment response.

Table 12.2 List of cancers with multiple biomarkers for measuring treatment response.

Cancer type	Biomarker	Description	Clinical application	References
Breast cancer	HER2	Protein overexpression linked to aggressive cancer	Monitors response to HER2-targeted therapies	Swain et al. (2023)
	CA 15-3 / CA 27–29	Tumor markers elevated in breast cancer	Used to monitor treatment response and recurrence	Lin and Genzen (2017)
	Circulating tumor cells (CTCs)	Cells shed into the bloodstream from primary tumors	Monitors treatment efficacy and disease progression	Kabel (2017)
	ESR1 mutations	Mutations in estrogen receptor gene	Monitors resistance to endocrine therapy	Kabel (2017)
Prostate cancer	Prostate-specific antigen	Protein produced by prostate cells, elevated in cancer	Monitors response to treatment, including surgery and hormone therapy	Ilic et al. (2018)
	CTCs	Cancer cells detected in the bloodstream	Indicates disease progression or response	Cieślikowski et al. (2021)
Lung cancer	EGFR mutations	Mutations in the EGFR gene affecting response to targeted therapies	Monitors response to EGFR inhibitors	Bethune et al. (2010)
	ALK rearrangement	Gene fusion that predicts response to ALK (Anaplastic lymphoma kinase) inhibitors	Used to monitor targeted therapy effectiveness	Cognigni et al. (2022)
	PD-L1 expression	Protein that inhibits immune response, is elevated in some lung cancers	Assesses response to immunotherapy	Yu et al. (2016)
	Circulating tumor DNA (ctDNA)	DNA fragments released by cancer cells into the blood	Tracks mutations and treatment response	Li and Liang (2020)

(*Continued*)

Table 12.2 List of cancers with multiple biomarkers for measuring treatment response. *Continued*

Cancer type	Biomarker	Description	Clinical application	References
Colorectal cancer	KRAS mutations	Mutations that impact response to EGFR inhibitors	Guides treatment decisions and monitors efficacy	Rahman et al. (2021)
	CEA	Protein elevated in colorectal cancer	Monitors treatment response and detects recurrence	Hall et al. (2019)
	ctDNA	Provides insights into mutation status and therapeutic response	Tracks emerging resistance mutations	Loft et al. (2023)
Ovarian cancer	CA-125	Glycoprotein elevated in ovarian cancer	Monitors chemotherapy response and recurrence	Charkhchi et al. (2020)
	HE4	Protein marker used alongside CA-125	Assesses treatment response and disease status	Chudecka-Głaz et al. (2023)
Pancreatic Cancer	CA 19-9	Carbohydrate antigen elevated in pancreatic cancer	Monitors response to therapy and disease progression	Ballehaninna and Chamberlain (2011)
	CEA	Monitors pancreatic and other gastrointestinal cancers	Used to evaluate therapeutic effectiveness	

12.6 Challenges and limitations in biomarker monitoring

One of the most significant challenges in cancer biomarker monitoring is the inherent heterogeneity of tumors. Tumors are not homogenous masses but are composed of different subclonal populations, each potentially exhibiting distinct genetic, epigenetic, and phenotypic characteristics. This heterogeneity means that a biomarker identified in one part of the tumor may not be representative of the entire tumor or other metastatic sites. Analytical sensitivity and specificity are crucial for the effective use of biomarkers in cancer monitoring. Sensitivity refers to the ability of a test to correctly identify patients with the disease (true positives), while specificity refers to the ability to correctly identify those without the disease (true negatives). In cancer biomarker monitoring, achieving high sensitivity and specificity is challenging due to the complex and variable nature of tumors. Low sensitivity can lead to false-negative results, where the biomarker fails to detect the presence of cancer, potentially delaying diagnosis or treatment adjustments. Achieving standardization is challenging due to the variability in assay design, sample handling, and data interpretation. Different laboratories may use different protocols, reagents, or instruments, leading to significant variability in results. Moreover, the lack of universally accepted guidelines for biomarker assay validation can further complicate the standardization process.

12.7 Conclusion

Cancer biomarkers have become essential tools in modern healthcare, playing a pivotal role in the diagnosis, prognosis, and monitoring of therapeutic responses in cancer management. They offer critical insights into disease biology, enabling early detection and the assessment of treatment efficacy. However, the inherent heterogeneity of tumors and the challenges in achieving high sensitivity and specificity in biomarker assays present significant obstacles. Advanced technologies, such as liquid biopsy, NGS, and mass spectrometry, have greatly enhanced the detection and quantification of these biomarkers. Despite these advancements, standardization across laboratories remains a challenge, highlighting the need for universally accepted guidelines and robust validation protocols. Addressing these challenges will be crucial in fully realizing the potential of cancer biomarkers in personalized medicine and improving patient outcomes.

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CHAPTER

Ethical considerations in biomarker research and application

13

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13.1 Introduction

Biomarkers have been differently defined and explained in many works of literature. In 1998 the National Institute of Health defined biomarkers as a biologically active substance that are used to evaluate normal biological processes, pathogenic processes of certain disease progression, and pharmacological effects of any specific therapeutic advancement. The World Health Organization has defined a biomarker as a substance or process that can be calculated in the human body, and it will help us to identify any ongoing disease progression or outcome. On the other side, many researchers have justified their ideas related to biomarkers as they are products of cellular, biological, and molecular changes that can be quantitatively analyzed from human cells, tissue, and fluids (Fathi et al., 2014). Recently, molecular biomarkers are very important and actively used in clinical research and clinical practice. Biomarkers play an important role in forecasting the future state of any patient's condition. There a two major types of biomarkers: predictive and prognostic biomarkers. The role of predictive biomarkers is usually in understanding of response of treatment to disease. and prognostic markers suggest the course of disease without any relevance to treatment (Buyse et al., 2011). Biomarkers have many applications such as diagnostic, therapeutic, treatment monitoring, and personalized medicine. The numerous biomarkers are defined in literature which can be useful for diagnostic approach. With all the present information on biomarkers, it is still challenging to select specific biomarkers and design diagnostic pathways (Califf, 2018). The concept of the development of biomarkers significantly influences personalized healthcare as well as medical interventions. Based on the use of biomarkers with their advanced characteristics brings an expansion in the field of medicine for both patients and diseases. The booming development of biological markers creates a lot of prospects in precision medicine and predictive medicine (Al-Dewik et al., 2022). Considering the growing demand for biological markers in different fields of medicine, it is now mandatory for clinicians and other health professionals to use such an approach by using high-quality clinical practice guidelines and ethical

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approvals. The guidelines and reporting standards should be appropriate for various aspects such as basic research, potential use for the same, efficacy efficiency, and effectiveness. It should be advised to each associate to improve and update in a timely manner (Horvath et al., 2010).

13.2 Informed consent in biomedical research

The fundamental concept of Informed consent is to explain and take permission of patient or research participants by explaining the procedure in which they are involved. To avoid unnecessary legal circumstances, it is a mandatory protocol to follow before proceeding with any scientific application associated with them. It's a kind of legal bond between participants and researchers in regard to safety-related aspects of study in terms of any injury or loss. The key elements of informed consent are the subject information form (SIF) and the informed consent form (ICF) (Chatterjee & Das, 2021). Research informed that consent also reflects the right to self-determination, it is known that everyone will take their own responsibility if they are involving themselves in medical procedures. This is normally considered during the use of any biological material from their body, that is, the use of DNA for any biological study. For others, they have different ways of getting permission from participants. Informed consent is usually obtained after sharing information with patient participants (Kegley, 2004). This approach is not only limited to the involvement of participants but also linked with biorepository where there is a huge demand for quality samples for clinical research and personalized medicine. Nowadays, with enhanced technology in medicine and an elevated need for analytical demand, the need for high-quality specimens is also required. The activities of biobanking are also affected by quality, ethical considerations, sustainability, and access to a good range of biological samples (Ahmed, 2011). A certain level of risk is associated with the selection of particiapants and their informed consent among choosing populations such as minors, cognitive impaired individuals, and prisoners. Originally selection of individuals has focused on two levels: socially and individually. To provide social justice it's been advised that investigators select as per order of preference to select class of subjects. (i.e., adults before children) and in certain conditions some subjects are involved like prisoners and mentally challenged people (Kolarcik et al., 2022).

13.3 Privacy and confidentiality concerns

For quantitative risk assessment, biomarker-related data of individual participants is useful. Because of ethical considerations, the subject is not liable to provide consent for the distribution of data to third parties, other researchers, or any union or organization. Doing or promoting such practice led to an impact on his/her job,

duties, and being answerable to legal matters. It will affect the person's ability professionally, financially, and socially. Moreover, in case of a high level of study where government bodies and social stigma related to disease conditions are involved, the data is protected by privacy act as per nation. On the other hand, there are certain rules in which a person can use data for research that allows by privacy act of nation.

1. In case when investigators use them for statistical research only
2. The associates require it to check the level of quality service they have provided
3. The law matters are involved, and they require to release it for awareness
4. Any national bodies require them to store as a historical event
5. In case of court demand for any decision for medico legal case

These above-mentioned situations were eligible to get information under terms and conditions given by the freedom of information Act and their distribution would not affect anybody's privacy (Schulte & Sweeney, 1995).

13.4 Clinical translation of biomarkers

However, clinical use of biomarkers are happening in different ways based on the information they provide: (1) biomarkers that explain risk of developing disease: antecedent biomarkers. (2) Biomarkers use for screening of any disease purposes: screening biomarker. (3) The markers that help in diagnosis: diagnostic markers, (4) the marker which helps to detect severity of disease: staging biomarkers, (5) The markers which give information about disease progression: prognostic marker (Frangogiannis, 2012). According to the perspective of US Food and Drug Administration (FDA's) initiative has focused on effectiveness of advanced biomarkers into drug inventions expected to enhance development of therapeutics and personalized medicine. For healthcare sector implementation of biomarker production at clinical level is a big task through achievement of analytical process to clinical validation and the showcase of evidence-based practices is time-consuming, needed efficient manpower and financial capabilities. The concept of biological markers are implemented on various disease like diabetic neuropathy, osteoporosis, proteomics, rheumatoid arthritis (RA), which is worth having enough role in diagnosis and treatment (Tesch et al., 2010). Actually the clinical term "biomarker" we use is new but concept is quite available in tradition of medical sciences. So, the advanced technologies required to implement biomarkers come in need to provide various utilities. In medical product development it is now mandatory to pass it from regulatory bodies. Hence, the US FDA has established regulations for biomarkers and other medical products development (Mattes & Goodsaid, 2018). As per the regulations, biomarkers are required to underwent assessment of diagnostic accuracy and predictability in a series of study that

requires to conduct from phase 1 to phase 5 which performance shows exploratory phase to outcome phase (Jain & Jain, 2017).

13.5 Incidental findings in biomarker research

In context of biological marker research incidental findings are most important issues for ethical. The incidental findings are known as a finding that reveals participants information which is associated with participants' future health (Viberg et al., 2014). One survey study conducted by Gliwa et al. (2016) among Institutional Review Board (IRB) professionals to understand whether they promote sending Information of incidental findings during genetic studies. The study data reveals that majority of IRB professionals believe the researcher should start disclosing incidental findings (Gliwa et al., 2016). During studying with any biomarker, the investigators are required to maintain certain duties for IF's, and it will consist of five core responsibilities: (1) Plan for standard protocols, (2). Provide information about possibility of findings of IF during informed consent. (3) Handle discussing IF with care. (4) Take a deep dive into findings of IF before disclosing it, take expert advice if needed. (5) Sometimes it needs to be discussed with study participants (Wolf et al., 2008).

13.6 Genomic biomarkers and ethical concerns

Genetic study is a very reliable to get advancement in improving medicine and disease management across the healthcare sector. As genomic study deals with personal information of patients and their family that is associated with ethical issues, legal and social implication (ELSI). Including ELSI there are many other declarations and acts that are useful for ethical consideration and prevent for negligence such as Ashcroft (2008), Schmidt (2004), Snead et al. (2009), World Health Organization (2002), (Ascencio-Carbajal et al., 2021). In regard with this subjects, here I am mentioning about various disease condition in which it is having active research on biomarkers. (1) Alzheimer Disease: Biomarker use for this condition helps to diagnose early-stage neurodegenerative changes. And their clinical criteria consist of challenges related to social and ethically. The investigators have discussed and evaluated major ethical issues related to diagnosis biomarkers validity for AD (Porteri et al., 2017). (2) Cancer Biomarkers: Biological markers play a vital role for cancer treatment and diagnosis to improve survival rates. Markers are used for treatment of cancer, disease monitoring, drug resistance or drug response (Purkayastha et al., 2023). For cancer related drug approval and healthcare policies are required to show high cancer mortality. Because of cancer pathogenesis its very difficult prove right evidence in order to use cancer biomarkers. With all such challenges its affects

patient doctor relationships and future problem arise in health care decision-making abilities. To prevent such scenario its difficult to focus on ethical aspects of use of drugs that improves patient survival rate. Upon focusing such aspects it is now important to balance ethical concern and preventive policies (D'Abramo & Guastadisegni, 2012).

13.7 Ethical implication of AI and machine learning in biomarker research

Artificial intelligence (AI) and machine learning and deep learning have powerful impact on revolutionize biomedical research. It is known as that it can copy human understanding and intelligence by machine through trained algorithms and that principle can be used for biomarker discovery (Mikdadi et al., 2022). Such method include various screening tools added to cell phones, applications, smart watches, sensors at home or in cars, also at various medical setups or imaging institutes (Ford et al., 2023). AI is a gift in context of patient care, diagnosis and drug discovery for treatment of any disease in area of modern medicine. In daily practice, AI-powered system helps clinicians to win challenging situations. With AI driven technologies clinicians benefit with more accurate treatment plan and implemented for patients with long term disease with accuracy and precision, that is, AI algorithm can easily recognized malignant cells and other lesions in medical images with accuracy and sensitivity. This advance leads to early diagnosis and impact full therapy for medicine (Katwaroo et al., 2024). Main concern ethical issue with AI is how they are connected to taking informed consent from the patient? This question has raised much attention toward ethical issues. Its not only limited to consent but several other problem are still in from to overcome like safety and transparency, algorithmic fairness and biases, and data privacy. To overcome this aspects many improvements are required to establish in terms of ethical and informed consent issue in AI-driven healthcare (Gerke et al., 2020).

13.8 Ethical framework and guidelines for biomedical research

Over many decades, a lot of guidance on ethical research conducts float with humans and most of the guidance was established in negligence, that is, Nuremberg code is remembrance of Nazi's Physicians; the Belmont report is response of Tuskegee syphilis case. Like this there are many examples which has given births to ethical framework. Secondly, the guidelines for regulatory affairs was create for one single standard for all the nation like development of pharmaceutical for human use. The aim behind it to develop safe and efficient use of drug discovery. Which is

known as the declaration of Helsinki (Emanuel et al., 2008). The Biomarker Task Force was entrusted with creating suggestions to enhance the choices made about the inclusion of biomarker studies in early exploratory drug trials by the IDSC of the National Cancer Institute (NCI). In order to ascertain the procedures and difficulties associated with carrying out biomarker studies in clinical trials of novel medications in the early stages of development, the members of the Task Force examined biomarker trials, the peer-reviewed literature, NCI, and FDA guidance documents. They also surveyed investigators. This document offers sponsors and investigators recommended practices for including biomarkers into such trials, along with standard definitions and categories of biomarkers (Dancey et al., 2010).

13.9 Conclusion

Biomarkers are physiologically active chemicals that are employed in the assessment of pathogenic processes of disease progression, normal biological processes, and the pharmacological effects of particular therapeutic advancements. They have a wide range of uses in therapeutic, diagnostic, treatment monitoring, and personalized medicine in clinical research and practice. The discovery of biomarkers has a big impact on medical interventions and personalised healthcare, opening up new possibilities in predictive and precision medicine. Clinicians and other health professionals must employ ethical approvals and high-quality clinical practice guidelines to ensure ethical concerns in scientific research. A key idea is informed consent, which involves patients or study participants in the process and guarantees safety-related factors. SIFs and ICFs are important components of informed consent. Informed permission is crucial for biorepository operations, as high-quality specimens are needed for clinical research and personalised treatment. It also symbolizes the right to self-determination in biomedical research. It can be dangerous to choose participants and obtain their informed consent, particularly when dealing with groups like juveniles, people with cognitive impairments, and convicts. When choosing participants, researchers should prioritize social fairness and preference order while taking age, mental health, and cognitive impairment into account. Quantitative risk assessment can benefit from biomarker-related data, but it's crucial to take confidentiality and privacy issues into account. Data distribution to organizations or third parties by subjects is not liable, which may affect their employment, responsibilities, and legal requirements. Confidentiality.

The US FDA seeks to improve therapies and personalized medicine by concentrating on the efficacy of sophisticated biomarkers in drug discoveries. The implementation of biomarker manufacturing at the clinical level is a difficult undertaking that calls both effective human resources and financial resources. The US FDA has set rules for biomarkers and medical product development, and the idea of biological markers can be used to a few disorders. Because incidental

findings in biomarker research disclose participant information related to the study, they raise important ethical questions.

13.10 AI disclosure

During the preparation of this work the author(s) used ChatGPT in order to During the preparation of this chapter, the author utilized ChatGPT to enhance the language and readability of certain paragraphs. Subsequently, the authors thoroughly reviewed and edited the content as necessary and take full responsibility for the publication's content. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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CHAPTER

14 Challenges and future directions in biomarker development

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14.1 Introduction

A biomarker is a chemical or activity that may be objectively quantified to indicate normal biological processes, pathogenic processes, or treatment responses. Cancer tissue or serum (Hill and Levi, 1954) biomarkers include DNA, mRNA, enzymes, metabolites, transcription factors, and cell surface receptors. The cancer biomarker field aims to create reliable, cost-effective, and powerful detection and monitoring tools for identifying cancer risk and early detection. The analysis of biomarkers is critical to personalized cancer care. It undoubtedly provides information on the patient's tumor, risk assessment, likelihood of recurrence, and treatment effectiveness, as well as the best course of action for them. There are numerous potential benefits of integrating biomarkers and modern technologies into the healthcare system.

14.2 What makes a good biomarker?

Very few of the numerous intriguing indicators that are being added to the biomarker pipeline eventually pass the test and revolutionize medical practice over time.

A biomarker's clinical effectiveness is determined by several significant qualities, regardless of its intended purpose. First, the degree and stability of the correlation between a biomarker and the target outcome, or disease, determine the biomarker's clinical utility. For this association to demonstrate high sensitivity and specificity over a broad spectrum of patient populations, it must be validated in several trials. Second, a transformational biomarker offers significant novel data that complements or enhances current assays. Third, the utility of the biomarker is significantly increased by the assay's affordability, simplicity of interpretation, convenience of analysis, and accessibility. Fourth, a biomarker is only valuable when it aids in the patient management process for the doctor. The therapeutic

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significance of the marker is therefore significantly strengthened by proof that a biomarker-guided strategy improves patient outcome; this suggests that medical therapies are available to change the course or outcome of disease.

In certain situations (Perera and Weinstein, 2000), biomarkers that do not direct medical interventions may still be helpful because they offer patients psychological advantages (such comfort when a test comes back negative for a dangerous condition). Naturally, the prerequisites for success depend on how a biomarker is going to be used. To be useful, biomarkers for cancer screening in large healthy populations must have a high specificity and be inexpensive. In addition, the disease of interest must be treatable with an efficient medical intervention. On the other hand, since successive measurements are made on the same patient, a very high sensitivity might not be necessary for biomarkers that track the course of a disease or a patient's response to treatment. Moreover, since only sick people are tested, cost might not matter as much.

Several methods have been developed to detect biomarkers on cancer cells, such as polymerase chain reaction, enzyme-linked immunosorbent assay (ELISA), electrophoresis, surface plasmon resonance (SPR), surface-enhanced Raman spectroscopy, microcantilevers, colorimetric assay, electrochemical assay, and fluorescence.

14.3 Classification

Analysis of biomarkers is crucial for specific cancer treatment, as it provides insight into the patient's tumor, including assessment of risk, relapse rates, therapeutic efficacy, and recommended treatment options. Integrating (Johnson, 2001; Jain, 2010) biomarkers and new technologies into the healthcare system has numerous potential benefits.

Disease-specific biomarkers (Table 14.1) are classified based on their ability to diagnose disease, predict therapy response, determine efficacy, and monitor compliance. Disease-related biomarkers are classified as predictive, diagnostic, or prognostic. Therapeutic-related biomarkers can assess therapeutic efficacy and patient outcomes.

- Based on their attributes, biomarkers fall into three categories: imaging, molecular, and nucleic acid.
- Based on applications, biomarkers can be categorized into diagnostic, prognostic, staging, and pharmacodynamic categories.
- Based on genetic and molecular biology confirmation, biomarkers can be categorized into natural history, pharmacological activity, and surrogate markers.
- Based on molecular understanding cancer, biomarkers provide insight into cancer progression, kind, best treatment options, and relapse rates..

Table 14.1 Classification of biomarker.

S. no.	Levels of classification	Examples
1	DNA epigenetic	Promoter hyper-methylation, e.g., genetic suppressors of the prp20-1(GSP1), death-associated protein in lung cancer; p15, p16 in liver cancer
2	DNA endogenous	Mutations, e.g., nicotinamide adenine dinucleotide dehydrogenase
3	DNA oncogene	Mutation
4	DNA exogenous viral	EBV (Epstein-Barr virus) in NPC (nasopharyngeal carcinoma), Burkitt's lymphoma; HPV (human papilloma virus) in cervical cancer
5	RNA celol-based endogenous	Tissue-specific markers, e.g., PSA mRNA endogenous in prostate cancer, cytokeratin 20 mRNA in breast cancer
6	RNA cell-free	Circulating mRNA, e.g., tyrosinase mRNA in melanoma
7	RNA exogenous viral	Viral RNA, e.g., EBV-coded RNA in NPC

Table 14.2 Specimen obtained for investigation of cancer.

S. no.	Type of cancer	Specimen for investigation
1	Oral	Saliva
2	Pancreatic	Pancreatic juice
3	Prostate	Seminal plasma
4	Colorectal	Stool

14.4 Specimens for the investigation of biomarkers

A biomarker is present throughout the body and is not limited to a single tissue. The majority of medical professionals and scientists favor minimally invasive or non-invasive methods when collecting specimens for study. The kind of cancer (Table 14.2) being studied determines the type of sample being used. The most significant issue with biomarker evaluation is the variety of methods used for sample collection, sample storage, further processing, and assay platform variation when evaluating the biomarker of interest. The most effective way to get samples is to snap freeze in liquid nitrogen and remove the necessary quantity of tissue for processing all at once. It is imperative to prevent freeze-thaw cycles to minimize fluctuation in the results completely.

The analysis of biomarkers is critical to personalized cancer care. It undoubtedly provides information on the patient's tumor, risk assessment, likelihood of recurrence and treatment effectiveness, as well as the best course of action for

Table 14.3 Various measures and their potential effects on health care.

S. no.	Measures	Effect
1	Improved diagnostics	Can lead to early detection and intervention
2	Application of pharmacogenetics	May reduce adverse side effects
3	Increased numbers of safe effective treatments	Improve treatment efficacy
4	Positive outcomes for patients and health care systems	Decrease cost and timelines
5	New biomarkers	Will potentiate the speed of drug delivery Will increase the safety of drugs Will increase efficacy of drugs

them personally. There are numerous potential advantages of integrating biomarkers and modern technologies into the healthcare system (Table 14.3).

14.4.1 Limitations of biomarkers

The majority of cancer biomarkers are proteins, which has the drawback of not being cancer-specific. Although not all cancer types contain genetic alterations, genetic biomarkers are more accurate than many protein biomarkers. The process (Pepe et al., 2001) of finding new biomarkers is hampered by a number of factors, including sample collection, transportation, processing representative tissues, reference standards, assay method sensitivity and specificity, and postanalytical analysis (Sturgeon & Chapter, 2009).

This chapter highlights current developments in cancer biomarker identification in this study. Our objective is to present a thorough analysis that addresses the current obstacles and recent advancements in attaining elevated selectivity and sensitivity in the identification of various cancer biomarkers. Using some recent examples from our own work and from relevant studies in the literature, the development in cancer biomarker detection is reviewed separately for discussion purposes based on the category of cancer biomarkers.

1. **Methods based on ELISA:** The most effective technique for identifying proteins in physiological samples, according to a lot of individuals, is the enzyme-linked immunosorbent test (ELISA), which is still often utilized in routine clinical diagnostics. In traditional ELISA protocols, colorimetric or fluorescent readout signals are used to observe target protein binding to a specific recognition site. Even with the development of numerous innovative ELISA-based protein detection techniques, there are still major barriers

preventing their widespread application in point-of-care (POC) diagnostics (Scaros & Fisler, 2005).

2. **Electrochemical and electrical detection methods:** The necessity to maintain the highly specific responses obtained during target binding—which also produces a relatively less substantial change in characteristics—is one of the primary obstacles addressed by many label-free tests of protein biomarkers. As a result, one needs to read with sensitivity. The inherent high sensitivity and simplicity of electrochemical/electrical detection techniques can be successfully integrated with smaller hardware. They might therefore be the most practical, quantifiable, and commercially viable of all the low-cost protein presence diagnostic tests (Scaros & Fisler, 2005).
3. **Optical methods:** Because the signal is frequently visible to the unaided eye, optical techniques are quick and don't require any washing steps, they show promise for use in POC early cancer diagnostics (Scaros & Fisler, 2005). Examples of these techniques include fluorescent methods, SPR strategies, and colorimetric assays resulting from light adsorption or scattering. Scintist created a multifunctionalized multiwalled carbon nanotube-based rolling circle amplification colorimetric and chemiluminescent system for the detection of cancer protein biomarkers by combining protein binding, DNA hybridization and replication, and enzyme catalysis.
4. **Biomarkers for cancer proteins:** Proteins are widely recognized as vital macromolecules present in living organisms, acting as the building blocks for many functions such as energy storage, metabolism, and cellular regulation. A particular disease is often associated with abnormal or unique expression of a protein. Compounds generated by cancer cells or by other cells in response to malignancy are frequently found in protein biomarkers for cancer diagnosis. Protein indicators are primarily obtained from blood, and rarely from urine.

There are several major challenges when analyzing restricted protein cancer indicators. First of all, proteins cannot be "amplified" in the same sense as nucleic acids since they cannot duplicate themselves to exponentially raise their concentration for the purpose of detection. Second, because proteins are extremely sensitive to variations in pH, ionic strength, and temperature, it is more difficult to identify low concentrations of cancer protein biomarkers. Third, because of the abundant background of other proteins, it is very challenging to accurately identify the traces of cancer-related proteins in complex or unrefined biological materials. Sensitivity, specificity, and accuracy are therefore essential factors to consider while developing biosensors.

14.5 Challenges and future directions

There is a lot of promise because of the distinct correlation between genomic alterations in malignant cells and the emergence of neoplasia of biomarkers for

cancer (Hartwell et al., 2006) patients' early identification, diagnosis, disease subtyping, prognosis assessment, therapeutic response assessment, and recurrence screening. Unfortunately, the pace at which new cancer biomarkers are being introduced into clinical practice is still incredibly slow, even with significant funding in the field and early trials suggesting considerable potential. Wagner and Srivastava (2012) offer their viewpoint on the causes of the sluggish translation of biomarkers in neoplastic diseases.

The relatively tiny size of the early tumor, which might not be connected to detectable changes in biomarker levels in the blood, urine, or stools, presents intrinsic challenges in the development of useful screening biomarkers, as the manuscript emphasizes. Additionally, the writers stress the significance of interdisciplinary and multiskilled teams to combine various data kinds to create biomarker-based tactics that work. Regretfully, early promising data in the current research environment are frequently based on work done by a single laboratory with little resources to meet the goals. The manuscript offers a methodical methodology for the identification, confirmation, and validation of biomarkers that can be used to treat many other disorders in addition to cancer in order to avoid these problems.

14.6 Diabetes biomarkers

In their review, Lyons and Basu address the role of biomarkers in diabetes. The writers emphasize the well-established Hemoglobin A1c, or HbA1c, is used in diabetes treatment as a marker of the occurrence and intensity of hyperglycemia (Lyons & Basu, 2012).

A significant advancement in the field may be the creation and validation of novel metabolic biomarkers that reflect the biochemical effects of diabetes and the biomolecular changes brought on by persistent hyperglycemia. The identification and assessment of individuals with diabetes complications may benefit considerably from the use of tissue-specific biomarkers.

14.7 Renal disease biomarkers

To address the diagnostic and therapeutic problems in acute and chronic renal illness, new biomarkers need to be introduced immediately. Slocum et al. (2012) spoke on the intense efforts being made to create novel markers for acute kidney injury (AKI) to aid in early diagnosis, prognostic assessment, and therapy advice. First, two to three days following AKI, serum creatinine levels do not rise. The limited sensitivity of serum creatinine is a critical issue because therapy effectiveness depends on early diagnosis of renal damage. Second, the etiology and pathophysiologic underpinnings of the disease are not indicated by serum

creatinine, which is a somewhat generic sign of kidney impairment. Third, there is a limit to its capacity to forecast treatment response (Slocum et al., 2012).

The search is on for novel plasma and urine biomarkers that could offer additional information beyond what normal laboratory testing and clinical evaluation can offer. Ju and colleagues explain the tremendous potential of genetic biomarkers in the diagnosis and treatment of individuals with chronic renal illness (Ju et al., 2012). Genomic markers can be one or more DNA characteristics (such as single nucleotide polymorphisms, DNA modifications, haplotypes, or cytogenetic rearrangements) or RNA characteristics (such as RNA sequences and expression levels) that measure the expression, function, or regulation of genes.

14.8 Inflammatory bowel disease biomarkers

The application of biomarkers in inflammatory bowel disease (IBD) is reviewed by Iskandar and Ciorba (2012). While endoscopy, biopsies, normal laboratory testing, and clinical assessment are the main methods used in the diagnosis and assessment of individuals with IBD, there are a number of fecal and serum biomarkers currently in the market that may offer additional information. Inflammatory markers (such as CRP and fecal leukocyte markers) can be used to track the progression of the disease and help distinguish between IBD and noninflammatory causes of diarrhea.

To support medical professionals in diagnosis and treatment, new biomarkers are required. Characterization of such biomarkers may also offer significant pathophysiologic insights, given our incomplete understanding of the etiology of IBD.

14.9 Pulmonary disease biomarkers

The function of biomarkers in acute lung injury and acute respiratory distress syndrome (ARDS) is covered by Bhargava and Wendt (2012). Diffuse alveolar pneumonia during the exudative phase of ARDS A severe inflammatory reaction accompanied by the production and release of cytokines and chemokines is triggered by damage and loss of cellular integrity.38 Although several inflammatory cytokines have been investigated as possible biomarkers in ARDS patients, the data indicates that their prognostic power for the emergence of lung damage (Ware, 2006) is limited. Biomarkers for growth factor release, cellular proliferation, and pro-fibrotic activity may provide prognostic information during the proliferative phase of ARDS. Due to the intricacy of the pathophysiology behind acute lung damage, it might be necessary to combine biomarkers to obtain clinically meaningful information (Vij & Noth, 2012).

While forced expiratory volume in 1 second (FEV1) and other functional indicators are well-established predictors of prognosis in patients with chronic

obstructive pulmonary disease (COPD), there is no correlation found between them and the patient's clinical status or level of symptoms. Therefore biomarkers are required to not only identify pathophysiologic subgroups of COPD but also give data on disease activity and clinical course (Cooper, 2006).

To throw light on the molecular causes of a disease, biomarkers are most useful when they reveal information about the activation of particular pathways. Multimarker techniques that evaluate the main components of the disease's pathophysiology may be used to give clinicians the data they need for diagnosis, prognosis assessment, and therapy planning.

14.9.1 Liquid biopsy

Liquid biopsy has the potential to revolutionize the diagnosis and treatment of Gastric Cancer patients by allowing for early discovery, molecular study of the entire tumor, and MRD detection following surgical excision: better risk stratification and systemic therapy recommendations. However, the clinical application of liquid biopsy in Gastric Cancer presents various problems. Developing liquid biopsy for Gastric Cancer is challenging due to low sensitivity and specificity. Single omics data may not provide complete molecular information but can supplement and enhance our understanding of tumor features. Liquid biopsy samples have low analyte content, making multiomics research hard (Han & Lee, 2024).

14.9.2 Integration of multi-omics data

The latest sequencing technologies have made "multi-omics" data increasingly important to the area of cancer biology. The phrase "multi-omics" refers to a more recent method of analyzing data sets that combines many omics data groupings, including the transcriptome, metabolome, proteome, genome, and microbiome, during analysis. Quantitative analysis of multi-omics data and clinical features can reveal information about modifications in molecular levels and facilitate a more methodical and comprehensive comprehension of intricate biological networks. The real information flow from one omics (WC, 2004) level to another can be seen by using an integrated multi-omics method. It will therefore assist us in filling in and bridging the gap between the genotypic and phenotypic levels. In due course, it will improve the precision of cancer diagnosis, prognosis, therapy, and prevention. Owing to the abundance of available data, big data analysis and the multi-omics method are required to connect all the pieces. To help with the creation of individualized treatment plans and the forecasting of clinical outcomes, for instance, data on patient demographics, genetics, proteomics, radiomics, and microbiota can be combined (Subramanian et al., 2020).

By combining information from several omics fields (Cho & Cheng, 2007), such as transcriptomics, proteomics, metabolomics (Cho, 2010), and genomics,

researchers can gain a deeper knowledge of cancer biology. Combining these datasets may lead to discovering novel biomarkers and molecular signatures, improving therapeutic options and diagnostic accuracy.

14.9.3 Advances in high-throughput technologies

To find circumstances of interest, or hits, for additional research, high-throughput screening (HTS) is a process that involves methodically screening a large number of variables for a single, or a defined small set, response, or mechanism. An HTS experiment can yield enormous amounts of data, therefore to reliably extract insightful evaluations, process flow and information management of the data become crucial. All things considered, hit selection screening (HTS) (Edsjö et al., 2024) is an excellent technique for thoroughly examining a great deal of circumstances or chemicals within a resource or library. It may also be used to efficiently find hits and more accurately qualify and forecast responses for preclinical applications in objective tests.

Next-generation sequencing and mass spectrometry are two examples of high-throughput (HT) technologies that enable the rapid analysis of large-scale datasets. These technologies facilitate the identification of biomarkers with enhanced sensitivity and specificity, leading to more precise cancer detection and surveillance (Edsjö et al., 2023).

HT approaches can show prospective new lines of inquiry, give researchers a more comprehensive understanding of biological reactions, and make it possible to identify "hit" compounds that can be further investigated in mechanistic experiments. We can anticipate quicker and less expensive drug development pipelines as well as improved yield in directed cell differentiation methods, which will improve personalized medicine and improve therapeutic results, as these systems become more physiologically relevant and repeatable.

14.9.4 Application of artificial intelligence and machine learning algorithms

These techniques can assist in the analysis of complex biomarker data and the identification of patterns that may not be obvious to human observers. Artificial intelligence (AI) can help in the identification of personalized therapeutic targets, the finding of biomarkers, and the prediction of therapy response, all of which can result in more accurate and focused cancer treatments (Singh et al., 2023).

AI methods, in particular machine learning (ML) and deep learning, have propelled revolutionary advancements in pharmaceutical research, surmounting obstacles to drug creation with unparalleled speed, increased effectiveness, and increased productivity and cost-effectiveness. Drug design, virtual screening, and drug-target interaction modeling are all aided by AI (Paul et al., 2021). AI is therefore used in a number of drug development processes, including as lead

optimization, toxicity prediction, target identification, hit identification, absorption, distribution, metabolism, elimination, and drug repositioning.

There are the several methods utilized in target identification and validation with artificial intelligence: (Köchert et al., 2024) methods driven by statistical analysis—these use omics data, such as Mendelian randomization (SMR) based on summary data and genome-wide association studies, to identify prospective target genes associated with disease. AI helps identify acceptable patient demographics and possible medication molecules, resulting in more effective and focused therapies. AI also helps with data mining in the real world, therapeutic medication monitoring, and improving the design and analysis of clinical trials. AI facilitates personalized therapy and boosts productivity in the pharmaceutical sector by streamlining procedures and enhancing decision-making. A big step toward bettering patient outcomes and developing healthcare is the use of AI in pharmacology.

Ethical and regulatory issues: It is critical to address ethical and regulatory issues, such as patient privacy, data sharing, and standardization of biomarker tests, as biomarker development advances. The responsible and moral application of biomarkers in clinical practice will be guaranteed by the establishment of policies and procedures.

14.9.5 Other future directions in biomarker development

- Predicting difficulties
- Providing personalized treatment advice
- Providing prognosis
- Improving the sustainability of drug development
- Enhancing the quality and safety of drug trials
- Reducing development costs
- Accelerating the approval process drastically
- Advancing personalized medicine

14.10 Conclusion

It is generally acknowledged that early cancer detection will enhance patient quality of life, lessen morbidity, and improve patient prognosis in addition to improving patient understanding. Over the past 10 years, developments in methods, analysis and techniques have made it possible to identify individual proteins from complicated proteome mixtures.

In summary, the quest for effective biomarkers in cancer diagnosis, prognosis, and treatment remains a complex and ongoing endeavor. Nevertheless, there is optimism for the future. Technological advancements, including the integration of multi-omics data, high-throughput techniques, and the emergence of liquid biopsies,

offer the potential for enhancing biomarker discovery and detection. Furthermore, applying AL and ML algorithms can boost the precision and effectiveness of biomarker assessments.

To overcome these challenges and make significant progress in biomarker development, continued research, collaboration, and standardization efforts are crucial. By addressing these obstacles and exploring new avenues, we can pave the way for more accurate, reliable, and personalized cancer diagnostics and treatments.

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CHAPTER

15 Success stories: impactful applications of cancer biomarkers

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15.1 Introduction

Biological markers of cancer are quantifiable signs found in the body that indicate the existence, stage, or advancement of cancer (Pal et al., 2022; Sadighbayan et al., 2019). Biomarkers refer to macromolecules, including proteins, nucleic acids, or metabolites, that are present in blood, urine, or tissue samples. In the realm of oncology, they are crucial since they help to timely identify, diagnose, predict, and monitor cancer (Das et al., 2023; Elkhader & Elemento, 2022). Under the framework of tracking treatment response, biomarkers offer important insight into the effectiveness of cancer treatment. They help doctors decide if the cancer is responding to treatment, preserving stability, or progressing. Making wise decisions on the continuation, adjustment, or change of therapy plans depends on this knowledge (Hesso et al., 2023; Jamil et al., 2022). For instance, the concentrations of particular biomarkers can be measures of how effective immunotherapies, targeted treatments, or chemotherapy are. The decline in biomarker levels could suggest how well the treatment reduces the cancer load. On the other hand, an

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increase in biomarker levels could point to either the evolution of the illness or the emergence of resistance to the current treatment strategies (Jiang et al., 2023; Kann et al., 2021). Personalized medicine relies a lot on biomarkers because they let medicines be tailored for each person based on their own biomarker. This way, it cuts down on unnecessary side effects and boosts treatment effectiveness. For instance, showing specific genetic defects in cancer cells can steer the use of targeted medicines designed to hit those defects. Biomarkers also help divide patients into different risk groups, influencing the dosage and follow-up strategies. By watching biomarker levels over time, doctors can catch relapse or progression sooner than with regular imaging methods, which improves patient outcomes (Martinez et al., 2022; Normanno et al., 2022). So, by giving essential data for tracking medication success and guiding personalized treatment plans, cancer biomarkers are really key in modern oncology (Sudhi et al., 2023). They cover early detection and ongoing monitoring, proving their worth in-patient care and treatment success Fig. 15.1.

15.1.1 Importance of monitoring treatment response

In cancer treatment, it is very important to keep an eye on how well the treatment is working. It makes sure that drugs for cancer are going to the right tumor, which helps them do better. Using biomarkers to check how well a treatment is working gives doctors quick and useful information about how well a therapy is working (Anstee et al., 2022; Dubois et al., 2021). This lets them change the treatment plan quickly if they need to. When doctors look at differences in biomarker levels, they can tell if a cancer is getting bigger, smaller, or staying the same. This helps you choose whether to keep the treatment you're already getting, try a new one, or add something else. Doctors can avoid bad side effects and switch to better treatments faster if they notice a bad reaction early on. Also, the best way to find minimal residual disease (MRD) is to keep an eye on how well the treatment is working. This means that there are still some cancer cells after the first treatment. Scientists can quickly treat MRD when they use biomarkers to find it. This lowers the chance that the cancer will come back and increases the chance of survival over the long run (Haleem et al., 2021; Kerr et al., 2021; Lan et al., 2024).

Finally, routine testing can detect if the malignancy is developing resistance to therapy, particularly with targeted and immunotherapies. The identification of biomarkers can detect alterations that may require adjustments in treatment. Conducting regular assessments of therapy efficacy is crucial for enhancing patient care through targeted modifications and increasing the likelihood of success. This results in enhanced efficiency and efficacy of cancer therapies in the long term. Broadly speaking, monitoring enhances patient care by enabling targeted adjustments. It enhances the likelihood of favorable results and contributes to more effective and successful cancer therapies (Pal et al., 2022; Pascual et al., 2022; Van Assche et al., 2022).

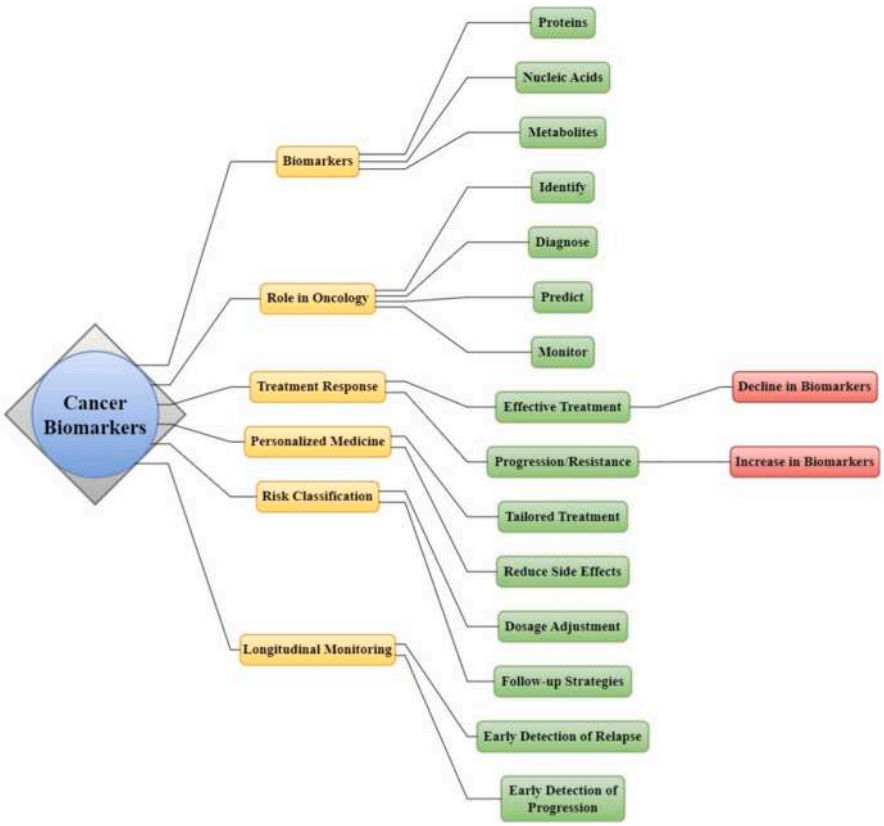

FIGURE 15.1

Overview of cancer biomarkers, including their role in identification, diagnosis, treatment response monitoring, and personalized medicine. Biomarkers guide therapy adjustments, risk classification, and early detection of relapse or progression.

15.1.2 The application of biomarkers in clinical settings

This chapter aims to investigate the efficacy of cancer biomarkers in monitoring treatment responses in actual medical environments. In the realm of individualized cancer therapy, biomarkers direct us in selecting the appropriate treatments (Blennow & Zetterberg, 2019; De Guire et al., 2013). They serve to determine the efficacy of treatments, detect first indications of cancer resurgence, and tailor therapies to the individual requirements of each patient. Our objective is to elucidate the specific role to which these biomarkers play in hospitals and clinics. We will examine the use of biomarkers to assess the effectiveness of medicines, detect any resistance over the course of treatment, and remain vigilant for any residual disease that may be concealed through time. Indeed, new technologies and

methodologies are always emerging, so enhancing the precision and dependability of these inspections. Issues include the necessity for standardized regulations and managing fluctuations in biomarker concentrations (Duffy et al., 2017; Kirkpatrick et al., 2021).

By highlighting these hurdles, we aim to spark ideas for solutions and show a path forward for future research and better use of therapies. So, get readyy for a dive into a brighter future where cancer treatment is even smarter and more tailor-made for each patient Fig. 15.2. The purpose of this chapter is to bridge the gap between biomarker research and clinical application by providing healthcare practitioners with clear guidelines on how to optimize biomarker use for patient

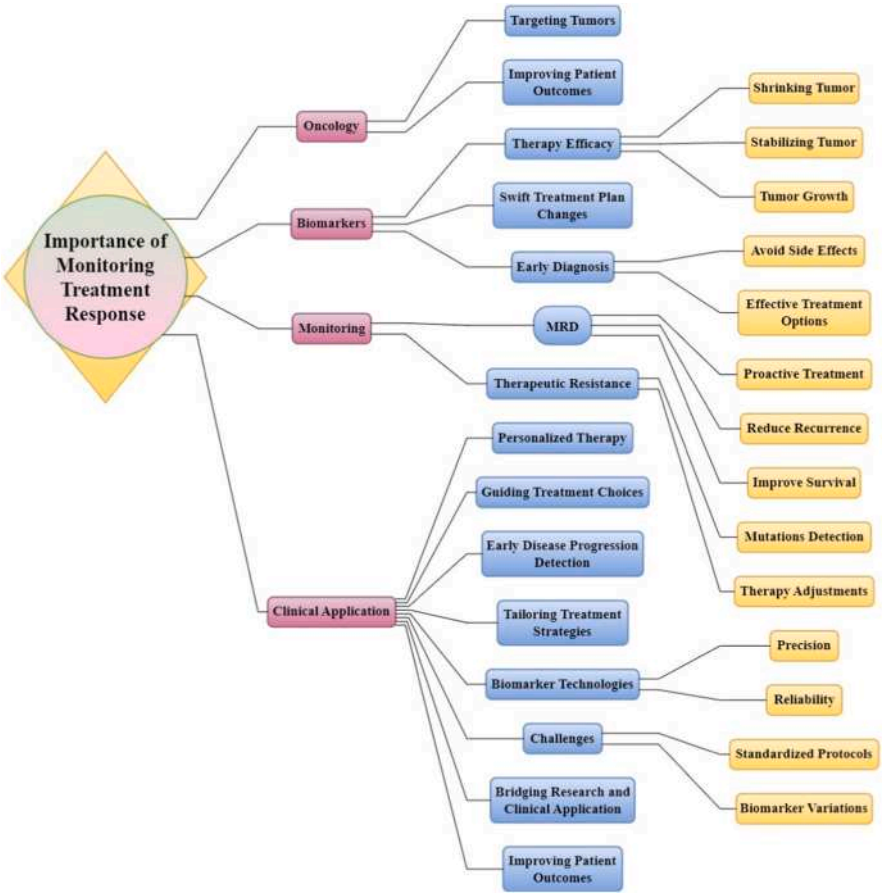

FIGURE 15.2

Overview of the importance of monitoring treatment response in oncology, emphasizing the role of biomarkers in assessing therapy efficacy, detecting minimal residual disease, and guiding personalized treatment strategies for improved patient outcomes.

management and successful therapy monitoring. This focused study aims to improve overall patient outcomes and contribute to the advancement of personalized cancer therapy (Kraus et al., 2011; Valentin et al., 2011; Wallis et al., 2010).

15.2 Cancer biomarkers: an overview

It is common to refer to biomarkers as biological markers. Biomarkers are observable signals that can be found in fluids, organs, or cells that indicate whether an organism is healthy or diagnosed with a disease. It is essential to have biomarkers in oncology. Moreover, they provide insightful knowledge of cancer biology as well as support disease diagnosis, prognosis prediction, and therapeutic response analysis. These instruments significantly help to customize cancer treatment and improve patient outcomes by means of efficient communication of important information that can direct therapeutic activities (Adamczyk et al., 2012; Barzaman et al., 2020).

In the field of cancer treatment, biomarkers fall into numerous categories according to their particular objectives and value. Diagnostic, prognostic, and predictive biomarkers are three main groups into which biological indicators fall. Diagnostic biomarkers are tests designed to identify medical disorders including cancer and other diseases. Moreover, they help to distinguish them from other diseases. Early cancer detection is essential since it improves general therapy outcomes and allows quick response. One often used diagnostic clue for prostate cancer is prostate-specific antigen (PSA), specific for that disease. By means of these signs, patients who require additional diagnostic procedures, such as biopsies or imaging-to validate the existence of cancer can be identified (Bensalah et al., 2007; Hoseok & Cho, 2015).

Regardless of the medication that is administered, prognostic biomarkers are able to forecast how the disease will proceed and the result. They contribute to the determination of survival rates, possibilities of recurrence, or the danger of progression of the illness. For instance, the quantities of particular genes or proteins in a tumor can indicate the degree of aggressiveness of the tumor or the likelihood that it will return following therapy. An example of this would be the levels of HER2 in breast cancer; high levels indicate a more aggressive type of the disease and a more hopeless outlook. Biomarkers that are predictive are able to foresee how a patient will react to particular therapy. Assist with the customisation of therapies by determining which medications are the most effective for each individual patient. The response of breast cancer patients to targeted therapy such as PARP inhibitors can be predicted based on whether or not they have a BRCA1 or BRCA2 mutation. Treatments can be tailored to the peculiarities of the cancer with the help of predictive markers, which allows for increased efficacy while simultaneously reducing the risk of adverse effects. The following categories of

biomarkers can be categorized according to their origin: Mutations in DNA or profiles of gene expression are examples of genetic biomarkers. These biomarkers act as indicators of genetic abnormalities that can lead to cancer. Measuring the number of certain proteins or patterns of proteins that show that cancer is present or spreading is called proteomic indicators. The metabolites found in bodily fluids or cells that are linked to cancer are called metabolic biomarkers. Epigenetic biomarkers are changes in DNA or histones that have an effect on gene expression but do not change the DNA sequences themselves (Manne et al., 2005; Pessoa et al., 2020; Staicu et al., 2020; Ullah & Aatif, 2009; Wittmann & Jäck, 2010). When you define and group biomarkers, you can see all the different ways they can be used in cancer treatment. By using these indicators, doctors can make more accurate diagnoses, guess how illnesses will progress, and change treatments, which improves oncology processes and patient care (Fig. 15.3).

15.3 The process of identifying and validating biomarkers

From initial discovery to clinical application, finding and developing biomarkers involve a complex, multi-step journey. Each stage plays a crucial role in ensuring that these markers effectively monitor cancer treatment.

15.3.1 Initial discovery phase

Biomarkers are found when scientists look at the basic processes of cancer up close and personal. Scientists look at a lot of different samples, like tumor tissues, blood, and pee, to find and tell the difference between healthy and cancerous cells. New techniques like genomics, proteomics, and metabolomics make this process of detection easier. Techniques like mass spectrometry, microarrays, and next-generation sequencing are used to make datasets pretty big. At the moment, the main goal is to find markers that can tell cancer cells apart and also reflect how well they respond to treatment. To find out if these markers are useful for reviewing treatment, preliminary studies using lab models like cell lines or animal tests are used (Bakker et al., 2021; Győrffy, 2021).

15.3.2 Validation phase

Once interesting biomarkers have been found, they need to be carefully tested to make sure they can be used in clinical settings. This phase has a lot of important steps: Validation of the analysis—At this stage, biomarker tests are closely checked for their accuracy, sensitivity, specificity, and ability to be repeated. It makes sure that the biomarker can be tested correctly in samples from real life (Harrison et al., 2020; Moqri et al., 2023).

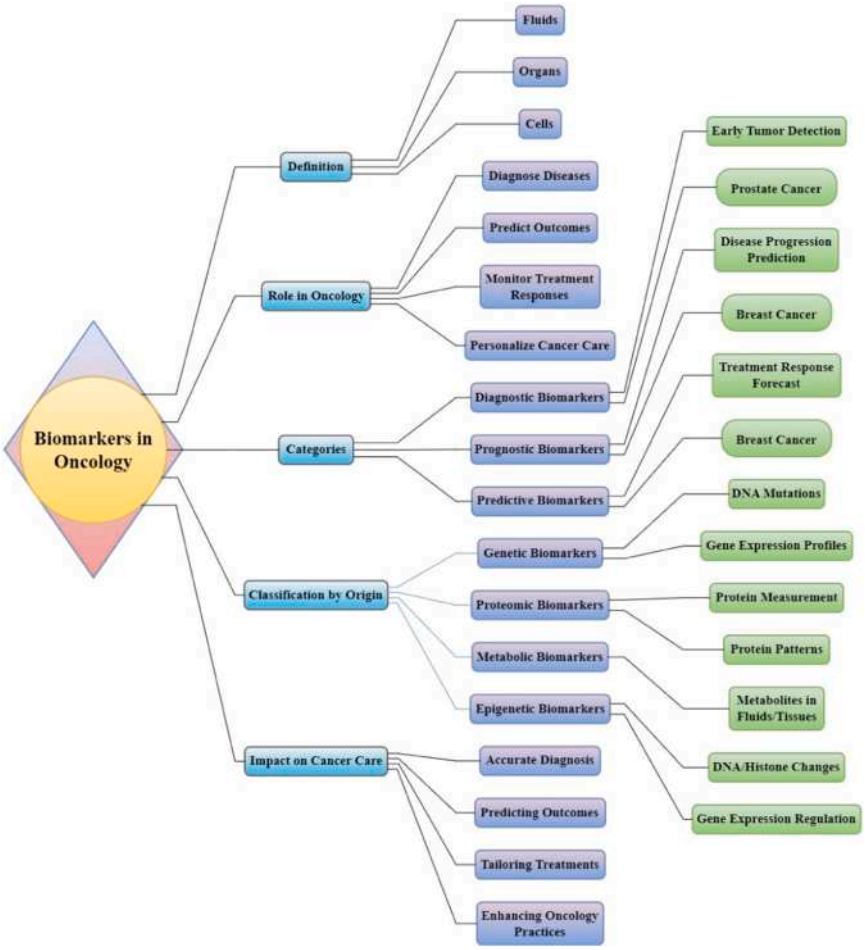

FIGURE 15.3

Illustration of biomarkers in oncology: Diagnostic, prognostic, and predictive biomarkers categorized by origin (genetic, proteomic, metabolic, epigenetic), highlighting their roles in cancer detection, prognosis, and treatment personalization.

15.3.3 Clinical validation

Researchers in this study look at the link between the biomarker and how well different groups of patients respond to treatment. The point of this study is to find out if the biomarker can predict or keep track of treatment outcomes in hospital settings. Larger and more diverse groups of patients are used to prove that the marker works for a wide range of cancers and treatments. Therapeutic Importance: A biomarker is clinically important if it can give useful information to improve patient care and achieve desired effects. It is important to figure out how it affects

decision-making, treatment choice, and patient results. This is the process of using clinical trials to guide treatments based on the biomarker and seeing how well they work compared to people who don't use it (Rim et al., 2020; Shu et al., 2020).

15.3.4 Regulatory approval

Biomarkers must be evaluated and given permission by the government before they can be used in normal clinical practice. This includes giving large amounts of quantitative data to governing bodies like the United States Food and Drug Administration (FDA) or United States Food and Drug Administration (EMA) to show that the drug is scientifically sound and works as a medicine. The method makes sure that the biomarker meets the set standards for safety, reliability, and effectiveness. Once biomarkers are approved, they are added to standard testing methods, expert training programs, and everyday clinical use. Post-market caution means keeping an eye out for any new problems. This is important for getting real-world data and making sure that development keeps going. To sum up, finding and making biomarkers is a hard and multifaceted process that includes everything from using modern methods to find them for the first time to fully validating them and getting regulatory approval for their therapeutic usefulness (Torres et al., 2020). The whole point of this method is to make biomarkers that are not only backed by science but also help doctors give better cancer treatment advice and, in the end, make patient results much better (Fig. 15.4).

15.4 Role of biomarkers in monitoring treatment response

15.4.1 Mechanisms of biomarker response

Biomarkers provide precise data that indicates the effectiveness of anticancer therapy. They enable physicians to observe the immediate impact of the therapy on a patient's tumor. This enables them to determine whether to continue or modify the therapy. Through an examination of various crucial processes, we can gain insight into the functioning of treatment by analyzing biomarkers (Alemohammad et al., 2022; Captur et al., 2020).

15.4.1.1 Mechanism of tumor response

Frequently, alterations in tumor biology align with observed biomarks as a result of treatment. An observed decrease in the amount of circulating tumor DNA (ctDNA) in the bloodstream would indicate that the treatment is effectively reducing the amount of cancer cells present. Chronic tumor DNA (ctDNA) refers to fragments of DNA that are released by cancer cells into the bloodstream, usually indicating the size and activity of the tumor. Typically, cell line DNA (ctDNA) levels decrease as therapy targets and reduces cancer cells, indicating a positive response to the

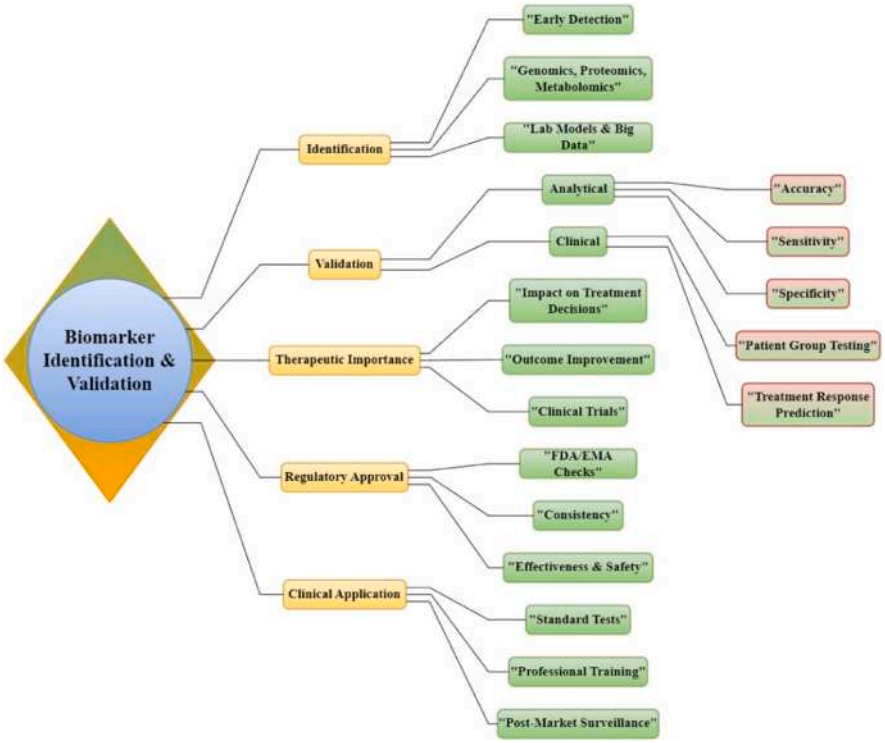

FIGURE 15.4

Flowchart of biomarker discovery and validation process: From initial identification in cancer research, through rigorous validation stages, to clinical application and regulatory approval, ensuring biomarkers' reliability and impact in cancer treatment.

treatment. Furthermore, alterations in specific protein biomarkers, such as HER2 in breast cancer or PSA in prostate cancer, might reveal variations in tumor activity and the effectiveness of targeted therapies (Cui et al., 2021; de Kock et al., 2021).

15.4.2 Molecular and cellular pathways

Biomarkers can indicate therapeutic efficacy by highlighting alterations in molecular and cellular pathways influenced by the therapy of the disease. Therapeutic interventions that specifically inhibit certain signaling pathways often result in subsequent changes in the expression of biomarkers linked to such pathways. If a drug successfully targets its intended impact, the activity or expression of associated biomarkers involved in that pathway will be reduced. Monitoring these advancements helps confirm that the therapy is generating the anticipated therapeutic outcomes at the cellular level (Hanjani et al., 2022; Kermali et al., 2020).

15.4.3 Restricted residual disease

Detection MRD refers to the presence of a small number of cancer cells that persist after initial treatment and have the potential to induce a recurrence. Biomarkers play a vital role in detecting this phenomenon. Detecting MRD early can be done with really sensitive biomarkers. These are better than usual imaging techniques. Special tests with high sensitivity to gene changes or protein markers can show cancer cells that imaging misses. Measuring MRD biomarkers is crucial. It shows relapse chances & helps decide if more treatment is needed (Kilgour et al., 2020; Saleh & Elkord, 2020).

15.4.4 Mechanism of resistance

Essential for monitoring treatment, biomarkers could indicate if medications no longer have effect. Variations in genetic biomarkers can highlight whether a tumor develops resistance to a given medication or changes in general state. New mutations or changes in the behavior of specific biomarkers, for example, would indicate that the cancer is not responding to the existing treatment regimen. Finding these resistance signals allows clinicians to change patients' treatment plans or consider different drugs before things worsen (Anstee et al., 2022; Sankar et al., 2022; Wolf et al., 2022).

15.4.5 Tailored treatment adjustments

Biomarkers are very important for figuring out how people react to treatment. With this knowledge, they let doctors decide on the best way to treat each patient. Biomarker levels can change, which can lead to changes in dosage, therapy, or the treatment plan itself in order to better fit the patient's unique reaction pattern. This customized plan makes it more likely that the best treatment results will be achieved while minimizing negative side effects. To sum up, biomarkers show how well a drug works in many ways, such as by showing changes in cancer biology and molecular pathways, finding metastatic regression disease, and checking for resistance (Bodaghi et al., 2023; Pérez-Ruiz et al., 2020; Saigusa et al., 2021; Shum et al., 2022; Sturm et al., 2022; Zhou et al., 2021). Biomarkers give doctors real-time information that helps them figure out how well a therapy is working, decide if it should continue, and change their methods to get the best results for the therapy and the patient's healing (Figs. 15.5 and 15.6).

15.5 Types of biomarkers for monitoring

The monitoring of cancer treatment response makes use of a wide range of biomarkers, each of which offers special information on the therapeutic

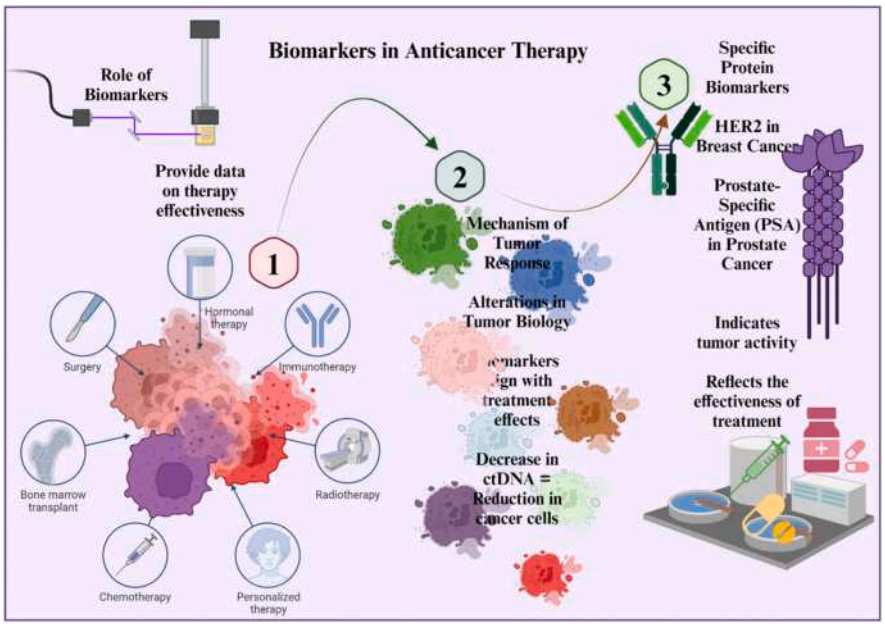

FIGURE 15.5

Overview of biomarkers in anticancer therapy, highlighting their role in monitoring treatment effectiveness, mechanisms of tumor response, and the impact of specific protein biomarkers like HER2 and PSA.

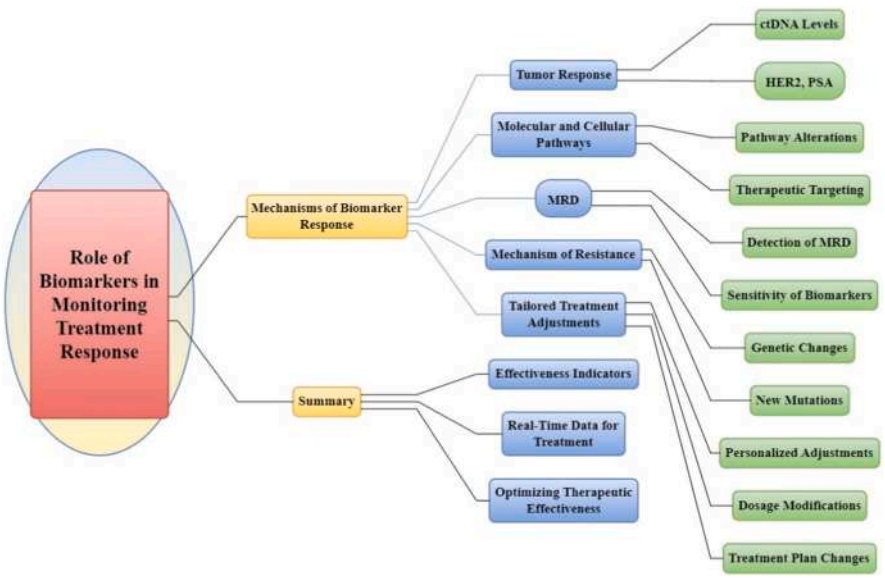

FIGURE 15.6

Biomarkers provide real-time insights into cancer treatment efficacy. They monitor tumor response, molecular pathways, residual disease, and resistance, allowing for tailored therapy adjustments and improved treatment outcomes.

effectiveness. Molecular biomarkers, imaging-based biomarkers, and cellular biomarkers are three categories under which biomarkers fall. Evaluating the effectiveness of therapy and guiding treatment decisions depend on all kinds of data (Alharbi, 2020; Batista et al., 2020).

15.5.1 Molecular biomarkers

Molecular biomarkers are important for figuring out how well cancer treatment is working because they give us a lot of information at the genetic, genomic, and protein levels. Some of these are DNA mutations, gene expression patterns, and copy counts. For example, finding certain changes in the BRCA1 or BRCA2 genes can let PARP inhibitors, a type of targeted treatment. Changing the amounts of certain oncogenes or tumor suppressor genes in the genome can show how well a treatment is working against cancer cells. It is helpful to keep an eye on these changes in real time when using markers like ctDNA. These markers, which can be found in a simple blood sample, tell us a lot about how tumors behavrk.

15.5.2 Proteomic biomarkers

Proteomic biomarkers look at tissues or body fluids to find specific proteins or forms of those proteins. PSA is only found in people with prostate cancer. In breast cancer, HER2 works similarly. By continuously monitoring changes in these protein levels, we can determine how well targeted treatments are functioning. If PSA levels drop after hormone therapy, it's an indication that the therapy is effective. These are small molecules involved in metabolic processes that cancer can affect. By examining blood or urine metabolomics, we can detect changes related to treatment response. Changes in metabolites like lactate or pyruvate may indicate modifications in tumor metabolism due to clinical intervention (Gutiérrez-Capitán et al., 2020; Karimi-Maleh et al., 2021; Kermali et al., 2020).

15.5.3 Biomarkers derived from imaging

Imaging-based biomarkers go hand in hand with genetic ones. They provide clear and detailed pictures of tumor size, volume, and other features. Some key radiographic biomarkers are CT scans, MRI scans, and PET scans. These helps track changes in tumor size and shape. The response evaluation criteria in solid tumors (RECIST) criteria check how tumors change on CT or MRI scans to see if treatment is working. Functional imaging biomarkers include advanced techniques like PET imaging with special tracers and DCE-MRI. DCE-MRI can look at tumor blood flow and how easily things move through the vessels. This can show changes in the tumor because of treatment. PET imaging uses tracers like fluorodeoxyglucose (FDG) to measure how active the tumor is. It can see how much a therapy is affecting the tumor's metabolism. In short, these imaging tools give a fuller picture

of what's happening with tumors during treatment (Ouyang et al., 2021; Sim et al., 2022; Vorkamp et al., 2021; Zaidi et al., 2020; Zheng et al., 2021).

15.5.4 Cellular biomarkers

Cellular biomarkers show how treatments affect cells. They let us see changes inside single cells. Cytometry flow is one way to check this. By measuring how cells scatter light & glow under fluorescence, it looks at both physical and chemical traits of cells. Using flow cytometry, we can measure how therapy changes cell surface markers or what's happening inside cells. You can also keep an eye on treatment results by looking at cell-free DNA (cfDNA) and exosomes-tiny packets released by cells. Pieces of DNA shed from cancer cells into the blood can be tracked this way too. The analysis of exosome content or cfDNA levels can provide insights into the mechanisms of tumor resistance and dynamics. Collectively, molecular, imaging-based, and cellular biomarkers are used to monitor the response to cancer therapy. Genetic, genomic, proteomic, and metabolomic markers, along with other molecular biomarkers, provide a fully comprehensive understanding of cancer biology and the effects of therapy. Although biological biomarkers offer insights into alterations at the cellular level, imaging-based biomarkers provide both visible and quantitative assessments of tumor modification (Anstee et al., 2022; Cecerska-Heryć et al., 2021; Khanmohammadi et al., 2020; Mani et al., 2021; Mostafa et al., 2021; Wu et al., 2021). Collectively, these few indications offer a comprehensive evaluation of the efficacy of therapy and guide personalized cancer treatment (Fig. 15.7).

15.6 Success stories: impactful applications of clinical relevance

The inclusion of biomarkers into clinical practice has fundamentally changed cancer and greatly affected clinical decisions-making. Precision and useful knowledge gained from biomarkers enable doctors to customise treatments, maximize patient outcomes, and properly control cancer. Several important factors highlight their therapeutic relevance: tailored treatment, tracking of illness development, early resistance diagnosis, better side effect control (Ahadi, 2020; Bime et al., 2020; Di Filippo et al., 2024; Ibrahim et al., 2023).

15.6.1 Tailoring therapist methodology

The way biomarkers let doctors customize medicines to every individual has a major influence on illness therapy. Looking at protein levels and genetic modifications helps one choose the correct therapy for that individual. Consider

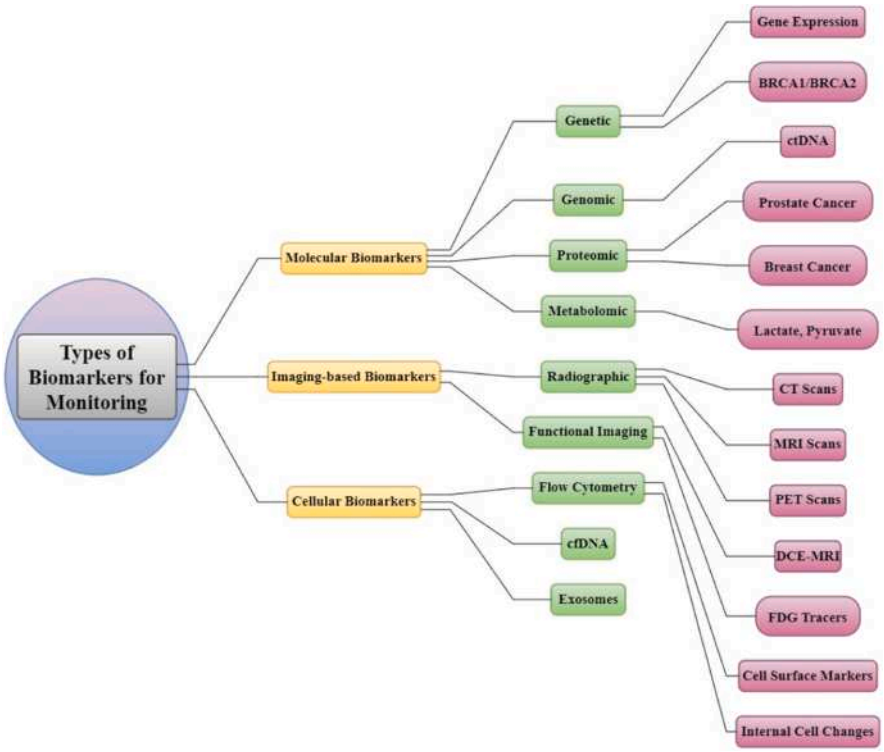

FIGURE 15.7

Overview of cancer biomarkers, including their role in identification, diagnosis, treatment response monitoring, and personalized medicine. Biomarkers guide therapy adjustments, risk classification, and early detection of relapse or progression.

breast cancer for one instance. High HER2 levels indicate to doctors they should prescribe medications like trastuzumab. These medications target high HER2 tumors most effectively. Patients with non-small cell lung cancer present another situation. Doctors can prescribe epidermal growth factor receptor (EGFR) inhibitors should individuals have mutations in the EGFR gene. This increases the probability of success of treatment. Thus employing biomarkers facilitates the matching of the treatment to the molecular composition of the tumor. This guarantees more exact and efficient cancer treatment (Jiao et al., 2020; Lino et al., 2022; Normanno et al., 2022; Pal et al., 2022).

15.6.2 Monitor disease advancement

All of tracking how a disease gets better or worse and verifying whether therapies are effective relies on biomarkers. Measuring ctDNA lets clinicians monitor cancer

without needing to conduct surgery. Generally speaking, declining ctDNA levels indicate that the treatment is working. Should they remain unchanged or increase, it could indicate either the disease is spreading or the medication is not as effective. These days, imaging indicators such as PET scans can directly depict what is happening. They show rapid images of a tumor's size and degree of activity. Doctors can determine if the treatment is working with these pictures and adjust if necessary. Regular viewing lets doctors adjust their strategies for maximum outcomes and steer clear of ineffective therapies (Sohrabi et al., 2022; Vincent et al., 2020).

15.6.3 Timely detection of resistance

Early drug resistance identification is much improved by biomarkers, which also enable prompt action. New mutations or changes in biomarketer profiles can indicate the emergence of resistance in a cancer to a given treatment. Early identification of such resistance lets doctors modify the course of treatment, look at new drugs, or mix several therapies approaches to get beyond resistance. Implementing proactive control strategies raises the possibility of disease control and patient survival (Barhoum et al., 2023; Gan & Roy-Chowdhuri, 2020).

15.6.4 Improved management of adverse effects

Biologic indicators can help in taking care of patients by finding out who might experience more side effects from their medicines. Some genetic biomarkers can show if someone is likely to have bad effects after chemo, such as organ damage or low white blood cells. Spotting risks early helps doctors change how much medicine to give, put in place preventive steps, or pick other treatments with fewer bad reactions. This customized way to handle side effects makes people stick to their treatment better and also makes their lives better (Glaab et al., 2021; Rodrigues-Ferreira & Nahmias, 2022).

15.6.5 Directional clinical trials and pharmaceutical development

Many times, biological indicators guide clinical studies and drug development. Patients can be arranged according to their likelihood of reacting to new. This increases the possibility of favorable outcomes and improves the quality of trial designs. Including biomarkers into research agendas helps researchers more precisely evaluate the efficacy of new medications. They can also identify patient groups most likely to gain from them. Clinical decisions depend much on biomarkers. They let for tailored treatment, monitoring disease development, early identification of resistance, and side effect management (Syedmoradi et al., 2021; Yekula et al., 2020). By means of biomarkers, cancer treatment becomes more exact and successful. Better patient outcomes and savings of resources follow from this. Research on biomarkers is projected to show their increasing utility in

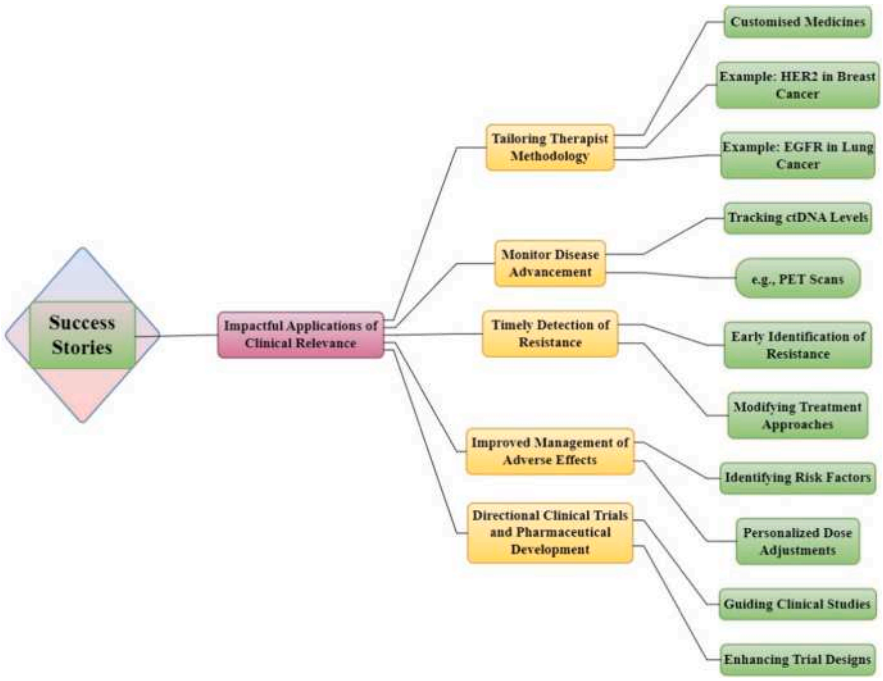

FIGURE 15.8

Impactful applications of biomarkers in clinical cancer practice, illustrating tailored treatment, disease monitoring, early resistance detection, adverse effect management, and guiding clinical trials, enhancing treatment efficacy and patient outcomes.

therapy, so altering cancer treatment and greatly boosting patient care much more (Fig. 15.8).

15.7 Success stories: case studies and clinical trials

Biomarkers have become increasingly important in monitoring treatment response in cancer patients, as they provide valuable analysis of therapeutic effectiveness and disease progression. The analysis of case studies and clinical trials demonstrates the expanding use of biomarkers in this sector, as well as their potential and limitations (Ampuero & Romero-Gomez, 2020; Barzaman et al., 2020; Glaab et al., 2021).

15.7.1 Case study 1

One well-known example is applying HER2, short for human EGFR, to treat breast cancer. Patients with HER2-positive cancer get specific therapies like trastuzumab

(Herceptin). Trials have shown that checking HER2 levels using immunohistochemistry or fluorescence in situ hybridization can guide treatment and predict how well it works. A major study in the *New England Journal of Medicine* found that patients with high HER2 levels who got trastuzumab had much better survival rates than those getting only regular chemotherapy (Ibrahim et al., 2023; Kilgour et al., 2020; Mann et al., 2021).

15.7.2 Case study 2

This case shows how biomarkers can be used to make it possible for more precise care and better clinical outcomes. Using an interpretative approach to look at EGFR in non-small cell lung cancer Changes in the EGFR help describe non-small cell lung cancer. EGFR mutations like exon 19 deletions or L858R point mutations decide which EGFR tyrosine kinase inhibitors (TKIs), like gefitinib and erlotinib, can be used. Like the studies in the *Journal of Clinical Oncology*, clinical trials have shown that checking for EGFR mutations is an important way to find people who will benefit most from targeted treatments. People with EGFR mutations reacted more generally to TKIs and lived longer without getting worse than people who did not have EGFR mutations (Normanno et al., 2022; Palmer et al., 2021; Sun & Benet, 2020; Wu et al., 2021).

15.7.3 Case study 3

Melanoma BRAF genetic alterations in BRAF V600E have significantly altered treatment approaches for melanoma. Clinical trials evaluating BRAF inhibitors such as vemurafenib and dabrafenib confirmed that patients with BRAF V600E-positive tumors saw substantial therapeutic benefits from targeted therapies. Studies published in *The Lancet Oncology* have shown that patients with BRAF mutations have superior response rates and survival compared to those treated with traditional chemotherapy. Therefore the use of BRAF mutation testing allows for more precise classification of treatment groups. These findings emphasize the important role of genetic testing in guiding personalized cancer therapy (Avgerinos et al., 2021; Boxer & Sperling, 2023).

15.7.4 Case study 4

PSA is a well-known marker, mainly used to check how prostate cancer treatment is going. PSA levels can be swayed by many things, like the side effects of drugs. But the trends in PSA levels are trusted to judge if the treatment's working and how the disease is moving along. A quick drop in PSA levels is tied, as noted in studies (like those in the *Journal of Urology*), to a good treatment response and improved clinical outcome. This case shows why keeping an eye on PSA is important for

managing the disease and checking early treatment results (Campuzano et al., 2021; De Haan et al., 2020).

15.7.5 Case study 5

Using liquid biopsies marks a major advancement in biomarker-based research. Liquid biopsies are less intrusive when blood tests analyze ctDNA. This aids in the search for left-over disease and helps to monitor therapy efficacy. Many clinical studies, including some in *Nature Reviews Clinical Oncology*, have shown that ctDNA levels can reveal tumor alterations, resistance mutations, and if the treatment is successful for many malignancies including breast and colorectal cancer. This method allows clinicians to monitor real-time therapy effectiveness and rapidly modify their course of action should need. Clinical research and case studies reveal that observing the effectiveness of treatment depends much on biomarkers. More study is required even if they enable therapies more appropriate for every patient to be better suited (Goldberg et al., 2020; Milne & Sin, 2020; Oliver et al., 2022; Scher et al., 2021). We must maximise the use of these indicators, overcome any challenges, and apply them for other forms of cancer (Figs. 15.9 and 15.10).

15.8 Highlight and notable biomarkers in current use in monitoring cancer treatment

The integration of biomarkers into daily drug therapy has significantly impacted clinical decision-making, and oncology has undergone a fundamental transformation. Physicians can maximize patient outcomes, customize therapies, and more effectively manage cancer by utilizing biomarkers' practical information and accuracy. The therapeutic significance of these factors is underscored by a variety of essential factors, including the enhanced management of side effects, the timely identification of resistance, the surveillance of disease progression, and the individualization of medicine (Barzaman et al., 2020; Campi et al., 2021).

Their flexibility to customize treatment is one of their most valuable tools in choosing a therapy. Molecular markers, such protein expression and genetic changes, guide the selection of some treatments most likely to be helpful for a given patient. For example, the detection of enhanced expression of HER2 in breast cancer patients directs the administration of HER2-targeted drugs such as trastuzumab, specifically formulated to combat tumors with elevated HER2 levels. Similarly, mutations in the EGFR gene discovered in patients with non-small cell lung cancer can result in the administration of EGFR inhibitors, therefore increasing the likelihood of a favorable therapeutic outcome (Kilgour et al., 2020; Ng et al., 2021). Through the alignment of treatment strategies with the molecular

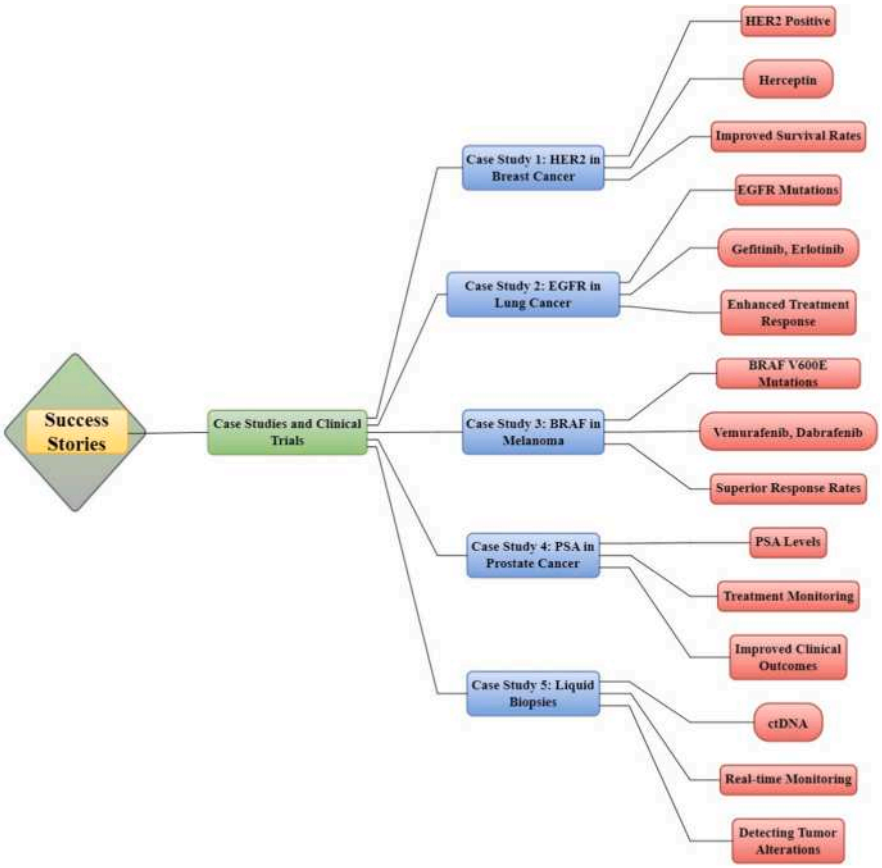

FIGURE 15.9

Case studies and trials demonstrating biomarker applications in cancer treatment: HER2 in breast cancer, EGFR in lung cancer, BRAF in melanoma, PSA in prostate cancer, and ctDNA in liquid biopsies.

characteristics of the cancer, biomarkers provide more precise and efficient control of the disease.

Biological markers determine the progression of a disease and assess the effectiveness of treatment. For instance, the non-invasive surveillance of tumor metabolism and therapy response relies on the quantification of ctDNA. A decrease in ctDNA levels, typically indicating a positive response to therapy, may suggest disease progression or insufficient treatment. On the other hand, numbers that are stable or rising would suggest the opposite. Imaging-based markers, like PET scans, also give doctors instant feedback on how the cancer is spreading and how well the metabolism is working. This lets them check how well the current treatments are

Cancer Type	Biomarker	Successful Impact
Breast Cancer	HER2/neu	HER2/neu overexpression has led to the development of trastuzumab (Herceptin), improving survival rates for HER2-positive patients.
Prostate Cancer	PSA (Prostate-Specific Antigen)	PSA testing has enabled early detection and management, significantly improving outcomes through timely intervention.
Lung Cancer	EGFR	EGFR mutation testing has led to targeted therapies like erlotinib and gefitinib, providing personalized treatment and better responses.
Colorectal Cancer	KRAS	KRAS mutation testing helps predict response to anti-EGFR therapies, optimizing treatment and avoiding ineffective treatments.
Ovarian Cancer	CA-125	CA-125 levels are used for monitoring treatment response and recurrence, improving management and follow-up strategies.
Melanoma	BRAF	BRAF mutation testing has enabled the use of targeted therapies like vemurafenib, leading to significant clinical responses.
Non-Small Cell Lung Cancer	ALK (Anaplastic Lymphoma Kinase)	ALK gene rearrangements are targeted by drugs like crizotinib, providing effective treatment options for ALK-positive NSCLC.
Kidney Cancer	VHL (Von Hippel-Lindau)	VHL mutation testing helps in understanding tumor pathogenesis and guiding personalized treatment for renal cell carcinoma.
Pancreatic Cancer	KRAS	KRAS mutation testing has contributed to the development of targeted therapies and better understanding of pancreatic cancer biology.
Gastric Cancer	HER2	HER2-positive gastric cancer patients benefit from targeted therapies like trastuzumab, improving treatment outcomes.
Chronic Myeloid Leukemia	BCR-ABL	BCR-ABL fusion gene detection has led to the development of tyrosine kinase inhibitors like imatinib, revolutionizing treatment for CML.
Hodgkin Lymphoma	PD-L1	PD-L1 expression is targeted by immune checkpoint inhibitors, offering new treatment options for patients with Hodgkin lymphoma.
Multiple Myeloma	M-protein	Monitoring M-protein levels helps in assessing disease progression and response to therapy, improving management strategies.
Testicular Cancer	AFP (Alpha-Fetoprotein)	AFP levels are used for diagnosis and monitoring of testicular cancer, guiding treatment decisions and follow-up.
Endometrial Cancer	MMR (Mismatch Repair)	MMR deficiency is associated with microsatellite instability, guiding the use of immunotherapy for endometrial cancer.
Soft Tissue Sarcoma	CD117 (c-KIT)	CD117 positivity in certain sarcomas helps guide the use of targeted therapies like imatinib, improving patient outcomes.
Head and Neck Cancer	HPV (Human Papillomavirus)	HPV-positive status is associated with a better prognosis and guides the use of specific therapeutic strategies in head and neck cancers.
Liver Cancer	AFP (Alpha-Fetoprotein)	AFP testing is used for screening, diagnosis, and monitoring treatment efficacy in liver cancer, aiding in better disease management.
Basal Cell Carcinoma	PTCH1	PTCH1 mutations are involved in basal cell carcinoma pathogenesis, aiding in understanding the disease and developing targeted treatments.
Acute Lymphoblastic Leukemia	T-cell receptor gene rearrangeme	nts T-cell receptor gene rearrangements are used for diagnosis and monitoring of treatment response in acute lymphoblastic leukemia.
Acute Myeloid Leukemia	FLT3	FLT3 mutation testing helps in predicting response to targeted therapies and guiding treatment decisions in acute myeloid leukemia.
Glioblastoma	IDH1/IDH2	IDH1/IDH2 mutations are associated with distinct clinical features and treatment responses in glioblastoma, guiding therapeutic strategies.
Bladder Cancer	FGFR3	FGFR3 mutations are targeted by specific therapies, improving treatment options and outcomes for bladder cancer patients.
Esophageal Cancer	HER2	HER2-targeted therapies improve treatment outcomes in HER2-positive esophageal cancer, providing effective management options.
Neuroblastoma	MYCN	MYCN amplification is a critical prognostic marker, guiding treatment decisions and providing insights into disease aggressiveness in neuroblastoma.

FIGURE 15.10

Illustrates the impact of key cancer biomarkers across various cancer types. Each biomarker enhances treatment efficacy by enabling early detection, guiding targeted therapies, and improving patient outcomes.

working and change them if they need to. With this kind of constant tracking, treatment plans can be changed to get the best therapeutic results while also cutting down on the number of medical procedures that aren't needed or don't work (Ahadi, 2020; Pal et al., 2022; Vincent et al., 2020).

Early drug resistance identified by biomarkers allows clinicians to begin therapy immediately. New mutations or changes in biomarkers, for instance, reveal that cancer is fighting a medication. Doctors might modify the course of treatment when they observe this quick resistance. To combat the resistance, they could investigate numerous medications or perhaps combine several treatments. These practical

techniques enable patients to maintain their condition more under control and live longer (Di Filippo et al., 2024; Ibrahim et al., 2023).

Biomarkers help us enhance how we care for patients who may have adverse drug reactions. Consider genetic biomarkers as an example. They can suggest serious consequences including organ damage or neutropenia after treatment. When these risks are detected early on, doctors can change dosages, give preventive care, or explore alternative treatments. This customised approach to reducing side effects not only improves patients' lives, but also aids in their adherence to their treatment regimen (Vincent et al., 2020).

Biomarkers are really handy in guiding clinical research and developing new drugs. They can group patients based on how likely they are to respond to treatments This makes good outcomes more probable and trial design better. Using biomarkers in trials helps scientists pick the right patient groups. It shows who will benefit and checks if new drugs work. By using biomarkers, doctors can choose the best meds for each person, watch how sickness progresses, find resistance early, and manage bad side effects (Pal et al., 2022). These tools help make cancer treatment work better and be more precise. This boosts patient results and saves resources. As we learn more, biomarkers should get even better for therapy. So, there'll be changes in treating cancer and improving care for patients Fig. 15.11.

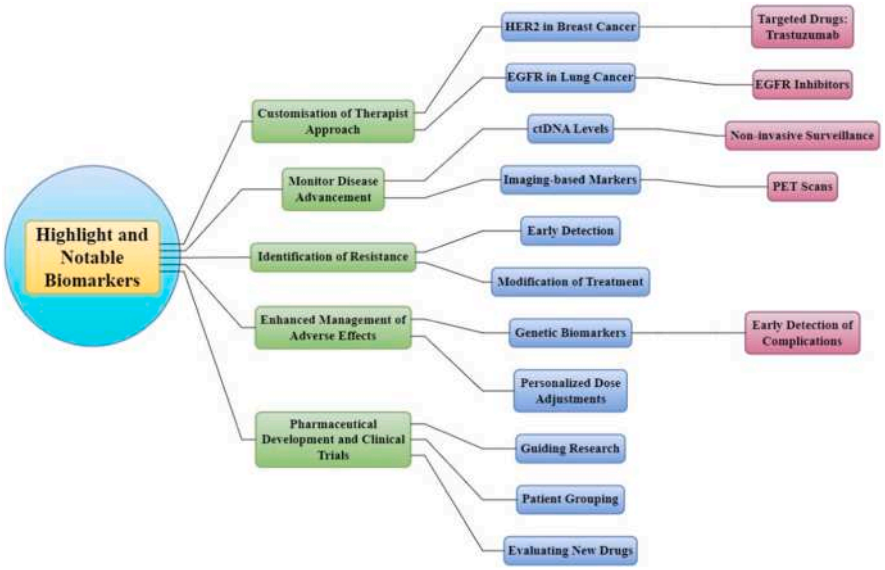

FIGURE 15.11

Overview of biomarkers in cancer treatment: customizing therapies (HER2, EGFR), monitoring disease advancement (ctDNA, PET scans), early resistance identification, managing side effects, and guiding pharmaceutical development and clinical trials.

15.9 Challenges in clinical implementation

15.9.1 Technical challenges

Assessment of therapy response using biomarkers poses various technical obstacles, such as problems related to standardization, sensitivity, and specificity. Both the precision and quality of biomarketer-based assays and clinical judgments are influenced by these issues. Sensitivity of a biomarketer, then, is essentially how well it can identify individuals with the disease or those undergoing treatment. Good sensitivity enables a biomarketer to display rapid reaction of a patient to treatment. However, achieving such high sensitivity is notalways straightforward. Low-sensitivity biomarkers can be challenging because their levels may not clearly reflect the effectiveness of a medication. This can result in missed opportunities to identify when the treatment is actually working, potentially causing doctors to mistakenly believe that the patient is not responding to therapy. This mess-up is particularly problematic in mixed forms of malignancies or when the biomarker finds it difficult to detect minute therapy changes (Alharbi, 2020; Bakker et al., 2021; Bodaghi et al., 2023).

15.9.2 Specificity

Conversely, specificity means a biomarker's ability to correctly identify individuals as not having disease or not responding to treatment. High specificity reduces false positives, where a biomarker wrongly reports disease presence or response to medication. A biomarker with low specificity might suggest a patient is responding to treatment when, in reality, another condition or factor is causing the observed changes. Attaining a high level of specificity is highly difficult, particularly in intricate conditions where biomarkers may interact with other physiological processes or diseases. It is crucial that biomarkers are not only sensitive but also relatively unique to the medicine or condition under investigation. Standardizing biomarkers presents yet another major difficulty in their monitoring (Karimi-Maleh et al., 2021; Kermali et al., 2020; Sturm et al., 2022; Torres et al., 2020).

15.9.3 Variability

Variability in biomarker measurement results from variations in test methods, laboratory approaches, and sample handling practices. In studies including protein biomarkers, for instance, differences in antibody quality, detecting techniques, and calibration standards can produce conflicting results. Furthermore, influencing genetic testing for mutation or alteration identification are changes in bioinformatics techniques and sequencing technologies. Standardizing these processes will help guarantee consistent and dependable biomarker values between several labs and clinical environments. Though they may be challenging and resource-intensive,

initiatives to provide consistent protocols and reference materials are ongoing (Wolf et al., 2022; Zaidi et al., 2020).

15.9.4 Preanalytical and analytical features

Analytical factors including assay sensitivity, detection limits, and dynamic range can also influence reliance of biomarketer measurements. Some biomarkers, for example, may be present in very low levels and demand very sensitive detection methods to exactly measure their levels. Among preanalytical factors that could generate variability affecting biomarker integrity and stability are sample gathering, storage, and processing. Samples must be treated consistently and tests must be confirmed for robustness under many conditions if we are to satisfy these challenges (Bodaghi et al., 2023; Karimi-Maleh et al., 2021; Zheng et al., 2021).

15.9.5 Integration of clinical practice output

Including biomarkers into routine clinical practice finally solves technological problems and ensures that the biomarker enhances patient therapy. These addresses confirming biomarkers in many patient populations, evaluating their clinical value, and developing therapy monitoring rules depending on them. Correct use of biomarketer-based monitoring systems depends on addressing these technical challenges (Kermali et al., 2020; Sturm et al., 2022; Wolf et al., 2022). Efforts to increase sensitivity, specificity, and standardizing as research advances and technology grow will be absolutely crucial in optimizing the use of biomarkers for effective and tailored cancer treatment (Fig. 15.12).

15.10 Regulatory and ethical considerations

Putting biomarkers to use in clinical practice to track how well cancer treatment is working comes with a lot of ethical and legal problems. Biomarker-based tests and therapies are harder to make, use, and accept because of this problem (Hesso et al., 2023; Hoseok & Cho, 2015; Lan et al., 2024).

15.10.1 Regulatory authority approval

Regulatory authorities such as the EMA and the FDA conduct rigorous oversight of biomarker-based testing. The biomarker test must be both safe and effective in achieving its intended objective. Comprehensive validation studies are an essential component of this crucial endeavor to ensure the diagnosis's relevance, reliability, and precision. These tests are repeatedly validated in a variety of clinical settings and with numerous cohorts to guarantee that the biomarker is accurately measured

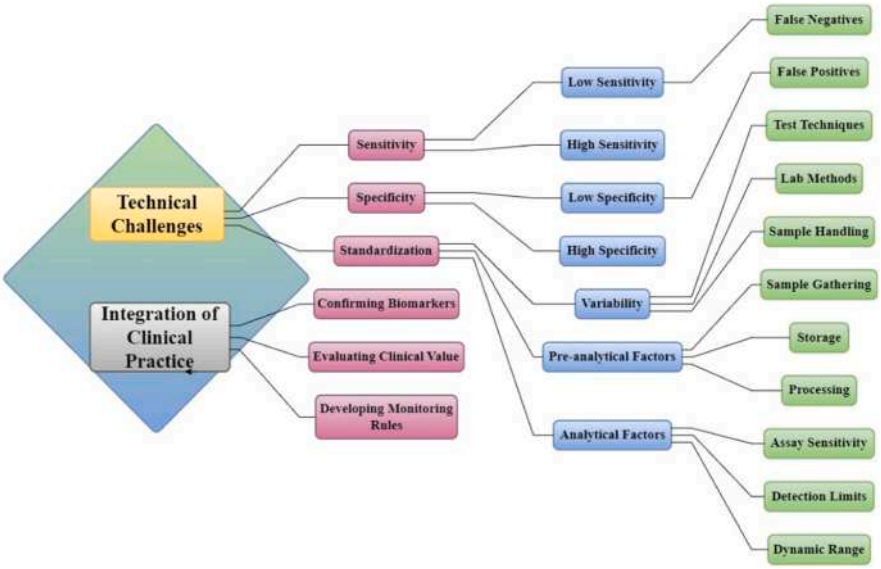

FIGURE 15.12

Challenges in clinical biomarker implementation: technical issues (sensitivity, specificity, standardization), regulatory hurdles, and ethical concerns. These factors impact biomarker accuracy, testing consistency, patient consent, and privacy.

on each occasion. In addition, they ensure that qualities such as sensitivity and specificity meet defined standards. This frequently means conducting a large number of clinical trials, which can be costly and time-consuming. Biomarker research is currently marked by rapid breakthroughs and the continuous introduction of new technologies. This keeps authorities alert, ensuring that regulations and standards are updated to reflect current legislation. Indeed, the situation is complex, but there is a lot of optimism. These advancements lead to better diagnostic tests and more effective future therapy for all individuals (Das et al., 2023; Ullah & Aatif, 2009; Van Assche et al., 2022; Wallis et al., 2010).

15.10.2 Ethical questions

People find the concept that biomarker tests could not be accessible everywhere intriguing. Because biomarketer-based testing can be somewhat costly, unequal access to these tests can exacerbate health disparities between persons of various income levels. Reducing the rising disparity in healthcare depends on everyone being able to access these tests. The informed agreement raises still another ethical dilemma. Patients must be fully informed on the goals, advantages, and any drawbacks of biomarketer tests. These covers understanding of their DNA information and privacy as well as how test findings might influence their

selected course of treatment. You have to make sure the patients can make wise decisions regarding biomarketer tests so honoring their right to be free (Hesso et al., 2023; Hoseok & Cho, 2015; Lan et al., 2024).

15.10.3 Privacy and confidentially

As part of biomarker tests, genetic material or other private biological data is often looked at. There are problems with privacy and secrecy because of this. It is very important to keep patient DNA and biomarker data safe to stop misuse and unauthorized access. In the United States, laws like the Health Insurance Portability and Accountability Act make it hard to make sure people follow the rules and deal with new privacy problems. Using biomarkers to guess how a disease will get worse or how well a medicine will work raises ethical questions. Patients and doctors must deal with the effects of knowing a biomarker points to a bad outlook, which can change mental health and treatment choices. A big social issue is how to balance the possible psychological effects on patients with the benefits of knowing more about their future. Using biomarkers to decide on treatment also brings up ethical concerns about patient liberty and the danger of relying too much on tests. Biomarkers can help doctors figure out a lot of things, but they should still use their own judgment and ask patients what they want. When you care for a patient well, you don't just make decisions based on biomarker data (Hesso et al., 2023; Hoseok & Cho, 2015; Martinez et al., 2022; Ullah & Aatif, 2009; Wallis et al., 2010). When creating and using biomarker-based methods, we need to be careful by putting social and legal concerns first. Following moral guidelines and setting up strong regulatory systems will assist in incorporating biomarkers into normal medical practice, safeguarding patients' rights and making sure that everyone gets excellent care (Fig. 15.13).

15.11 Discussion

Cancer biomarkers are a key part of personalized medicine because they show how well a treatment is working. How we care for people with cancer changes a lot because of it. Biomarkers, which can be genes or molecules, tell us a lot about a disease or how well a medicine is doing. This helps us make treatment plans that are more focused and work better. Biomarkers are a great way to keep track of therapy because they give real-time information about how the treatment is going. Imaging and tumor tests are two traditional methods that often give wrong information about the current state of the disease and give late feedback. Biomarkers, on the other hand, let less invasive means like blood tests keep track of how well treatment is working overtime. For example, looking at the amounts of ctDNA could give you instant information on how well a cancer treatment is working. It might even show resistance or return before pictures do. Biomarkers are another tool that can help

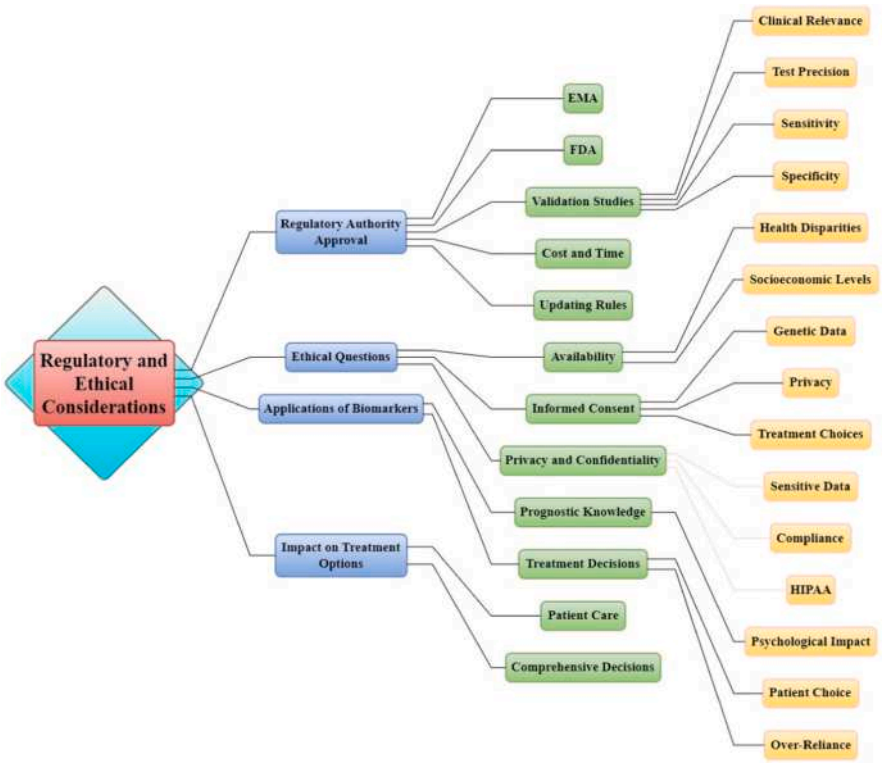

FIGURE 15.13

Challenges in integrating biomarkers into clinical practice: regulatory approval, validation, ethical issues (e.g., availability, informed consent, privacy), and impact on treatment options, including balancing utility and patient care considerations.

you figure out how harmful a medicine is. Some indicators can show that certain drugs are having bad side effects. This lets doctors change treatments to make them less dangerous. This personalized plan not only keeps patients safe, but it also makes sure they get the right dose with the fewest side effects possible, which improves the result of their treatment. Biomarkers can be hard to use in everyday clinical practice, though.

Biomarker levels change, but we need to make sure they are the same in all labs and systems. This variation could make it hard to correctly understand the results. Biomarkers like HER2 in breast cancer and PSA in prostate cancer, for example, can only be useful if they are measured accurately and tested under the same conditions every time. Assay results that don't match up or big differences in biology can cause different treatment choices. Another problem is that biomarkers can sometimes give false positives or negatives, which can change the treatment decision. Clinical confirmation is needed for biomarkers to be reliable and accurate.

To show that biomarkers can regularly predict treatment outcomes, a lot of testing needs to be done with a lot of different patient groups and clinical settings. Even with these problems, we are still finding and confirming new biomarkers. Multiplex biomarker panels test many biomarkers at the same time to get a full picture of how treatment works. New biomarkers are being made possible by progress in DNA and proteomics. We can now tell not only if cancer is present, but also what kinds of genes it has and what kinds of resistance patterns are starting to show up. Using biomarkers to track a treatment's reaction has many benefits, including getting information in real time, making sure that treatments are exactly what the patient needs, and better managing side effects. Although new research and technology are allowing biomarkers to be utilized more successfully in cancer treatment, assay standardization and biomarker accuracy remain issues that demand attention. More advancement in this field could result in more precise and successful cancer treatments, therefore enhancing patient results and providing more customized treatment options (Das et al., 2023; Hesso et al., 2023; Hoseok & Cho, 2015).

15.12 Conclusion

Individual response to treatment is being monitored using biomarkers, which is transforming cancer care. This provides another option for controlling and optimizing therapy. Biomarkers have become an excellent tool for following the course of therapeutic intervention due to their ability to provide real-time data on treatment success and the symptoms displayed by each particular patient. They assist clinicians in making quick assessments, changing their treatment plans based on the responses of specific patients, and increasing the overall effectiveness of treatment while decreasing the number of side effects. Personalized medicine, in which treatment procedures are tailored to the specific biological characteristics of each individual cancer patient, is becoming more widespread as biomarkers are used in clinical settings. This technology not only provides a more precise tool for medication evaluation but also addresses the issues associated with previous approaches, such as imaging, which may not always accurately reflect the current state of the condition. Furthermore, biomarkers enable early detection of drug resistance or relapse, allowing for faster intervention and perhaps better patient outcomes. However, employing biomarkers is not always an easy process. If we wish to maximise them, we must address concerns such as test standardization, biomarker variation control, and false results prevention. Constant research and the advancement of new technologies will assist to solve these issues, increase the accuracy of biomarketer testing, and demonstrate their dependability across a wide spectrum of patient groups. Despite ongoing hurdles, biomarkers hold significant promise for improving customised cancer treatment and surveillance tactics. Personalized cancer treatments, better patient outcomes, and advancements in oncology should all result from ongoing research and the use of biomarkers in

clinical settings. To find more accurate and tailored cancer treatments, biomarkers must first be discovered and then implemented in clinical settings.

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Author contributions

PT, SK, and PP contributed to the study's conception and design. PT, PP, and SK performed analysis the data. PT, SK, and PP wrote the original manuscript. MG, NK, AD, and ASK proofread it. All the authors have read and approved the manuscript for submission.

Declarations

The authors confirming that the work presented is original, has not been published elsewhere, and that all necessary ethical considerations have been followed, including proper attribution of sources.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this chapter.

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CHAPTER

16

Shaping future diagnostics: advances and innovations in cancer biomarker science

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16.1 Introduction

Cancer biomarkers are compounds or molecules with significant therapeutic value that show the onset, progression, or spread of cancer. These may consist of cancer-specific proteins, isoenzymes, nucleic acids, metabolites, hormones, or tumor cells that exhibit changed expression levels in malignant circumstances. Depending on whether they serve as prognostic, predictive, or diagnostic markers, they are classified (Hasan et al., 2019; Jayanthi et al., 2017; Sarhadi & Armengol, 2022). As "response modifiers" or "predictive factors," they can help determine which course of treatment is most likely to work for a certain patient. For instance, because of the correlation shown between the KRAS somatic mutation and a poor response to anti-epidermal growth factor receptor (EGFR) therapy, KRAS functions as a prognostic biomarker in colorectal cancer (Allegra et al., 2009).

Additionally, a biomarker can consist of various alterations, including gene expression, proteomic, and metabolomic signatures (Henry & Hayes, 2012). Comprehensive research into substances found in cancer tissues as well as in the bodily fluids of cancer patients has advanced the identification of cancer biomarkers. These compounds include proteins, hormones, and enzymes. (Passaro et al., 2024). These biomarkers are available for noninvasive and reproducible evaluations because they can be found in body fluids such as whole blood, serum, and plasma as well as excretions and secretions like stool, urine, sputum, or nipple discharge. Furthermore, tissue-derived biomarkers necessitate a biopsy or specialized imaging techniques for evaluation (Henry & Hayes, 2012).

These markers were primarily identified through immunological techniques, such as radioimmunoassay. The advancement in biological sciences has significantly propelled technological innovations. Over subsequent decades, this

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advancement has facilitated the development of sophisticated analytical methods, particularly in mass spectrometry (MS), and the creation of protein and DNA arrays (Passaro et al., 2024).

16.2 Type of biomarkers and advancements

16.2.1 Circulating DNA

Fragmentomics is a new field focused on studying circulating DNA (cirDNA), particularly for cancer screening, which was first introduced by Ivanov et al. (2015) to describe the analysis of cirDNA fragment size profiles. A portion of circulating DNA (cirDNA) consists of extracellular DNA circulated during cell death processes, particularly necrosis, phagocytosis, or apoptosis (Thierry, 2023). Using gel electrophoresis, early detection of cirDNA consists of fragments of approximately 100–500 base pairs (bp) in length (Jahr et al., 2001). In individuals with cancer, there are differences in cirDNA. There are more DNA fragments between 151 and 218 bp and fewer fragments below 150 bp as a result of these variations (Sanchez et al., 2021).

Over and above, the genome-wide map of nucleosome occupancy was identified and patterns pertaining to chromatin/nucleosome occupancy were validated with the aid of cirDNA sequencing. The footprint of transcription factor occupancy was identified by short cirDNA fragments. This technique can identify the cell types that contribute to cirDNA in pathological conditions such as malignancy (Snyder et al., 2016; Underhill et al., 2016). Mutant alleles frequently manifest as shorter cirDNA fragments. The majority of short cirDNA fragments are associated with nucleosomes. Furthermore, by using bioinformatics to isolate a certain group of fragment lengths, mutant cirDNA can be found. Through the application of this technique, cirDNA has been more sensitively detected in vitro and in silico conditions, allowing for the enrichment of cirDNA fragments with sizes ranging from 90 to 150 bp (Hellwig et al., 2018; Mouliere et al., 2018; Underhill et al., 2016).

16.2.1.1 cfDNA

16.2.1.1.1 Background

Since its discovery in 1948, the bloodstream has been shown to contain cell-free DNA (cfDNA), which is now recommended as a diagnostic tool for cancer patients (Diaz & Bardelli, 2014). While the total amount of cfDNA in the plasma and serum can vary among individuals, cancer patients typically exhibit higher average levels of cfDNA compared to individuals without cancer (Bennett et al., 2016). Hence, elevated levels of cfDNA can function as a valuable indicator of cancer across different tumor types, making it a promising biomarker for early diagnosis and disease monitoring. Consequently, it holds significant clinical potential in various aspects of cancer management (Czeiger et al., 2011; Paci et al., 2009).

Most cfDNA typically originates from stromal cells and leukocytes that are in good health. However, circulating tumor DNA (ctDNA), a portion of cfDNA produced from tumors, can also be present in cancer patients. The differentiation between healthy individuals and cancer patients can be based on the amount of cirDNA, which is estimated to be around 2000 haploid genome equivalents per milliliter in healthy people. In contrast, cancer patients typically have higher levels of cirDNA (Kilgour et al., 2020).

16.2.1.1.2 Detection

cfDNA can be detected by different methods. For instance, real-time polymerase chain reaction (PCR) was used by Paci et al. (2009) to quantify plasma DNA. While Czeiger and his team utilized a simple fluorescent test involving fluorescent SYBR Gold Nucleic Acid Gel Stain (Invitrogen) to detect the circulating cell-free DNA (CFD) in plasma directly, eliminating the need for DNA extraction. A 96-well fluorometer was used to measure the fluorescence at a 535 nm emission wavelength and 485 nm excitation wavelength (Czeiger et al., 2011). Moreover, A CC-tag technology utilizing methylated cytosine for the end repair reaction was later reported to facilitate the identification of urinary cfDNA in the plasma of bladder cancer patients. This was achieved by analyzing jagged ends through bisulfite paired-end sequencing, a method known as Jag-seq (Zhou et al., 2021).

Additionally, DELFIDNA evaluation of fragments for early interception: large-scale chromatin organization in several early-stage malignancies is assessed using genome-wide cfDNA fragmentation (Cristiano et al., 2019; Mathios et al., 2021).

Nevertheless, urine containing cfDNA has shown promise as a cancer biomarker. Urine samples from patients with progressing nonmuscle-invasive bladder cancer have shown higher amounts of cfDNA, as one prime example. Interestingly, in some cases, levels of cfDNA in urine were found to be high even when levels were low in plasma samples from the same patients. This highlights the value of urine-based cfDNA analysis as a noninvasive and potentially more sensitive method for detecting and monitoring bladder cancer progression (Birkenkamp-Demtröder et al., 2016).

16.2.1.1.3 Challenges for using in clinical practice

Certainly, before cfDNA can be widely adopted as a biomarker in clinical practice, several challenges need to be addressed. These include (Ralla et al., 2014):

Technical challenges Optimizing protocols across preanalytical, analytical, and postanalytical stages, involving enhancing procedures for sample collection, processing, storage, cfDNA isolation, quantification, mutational analysis, data processing, and interpretation.

Standardization Standardization of operating procedures across different laboratories and clinical settings to ensure consistency and reliability of results.

Data validation Validation of findings through large multicenter studies to assess the reproducibility, accuracy, and clinical utility of urine-based cfDNA analysis as a biomarker for cancer detection and surveillance.

Addressing these challenges is crucial for establishing the robustness and clinical validity of urine-based cfDNA biomarkers, paving the way for their widespread acceptance and integration into routine clinical practice for cancer diagnosis and management.

16.2.1.2 Circulating tumor DNA (ctDNA)

16.2.1.2.1 Background

In 2004 findings from Lo's team suggested that the majority of ctDNA molecules are between 145 and 201 bp in length, as determined using qPCR (Chan et al., 2004). Furthermore, ctDNA can be used to analyze mutations and copy number alterations, which are increasingly used to forecast responses to targeted medicines and assess the efficacy of treatment (Kilgour et al., 2020).

Even though ctDNA was first discovered in 1869, its actual use in practice has only now become possible (Capuozzo et al., 2023). Subsequently, Roche and AstraZeneca developed a ctDNA predictive biomarker as a companion diagnostic for their EGFR inhibitors. Recurrent somatic EGFR was reported in Western and Asian non-small-cell lung cancer (NSCLC) patients with approximately 15% and 40%, respectively. These mutations, often found in exon 19 (ex19del) and exon 21 (L858R), activate EGFR and lead to ligand independence (Pao & Chmielecki, 2010). Subsequently, in 2016, the cobas EGFR Mutation Test v2 was the first ctDNA-based companion diagnostic test approved by the FDA for detecting EGFR ex19del or L858R mutations from plasma (Kwapisz, 2017).

16.2.1.2.2 Detection

As demonstrated for EGFR mutation detection for NSCLC, ctDNA analysis can function as a sensitive liquid biopsy in tumor types with recurrent driver mutations, such as BRAF-mutated melanomas or KRAS mutant pancreatic cancers. With sufficient input DNA, RT-PCR and ddPCR techniques can detect minute numbers of variations with great sensitivity (variant allele frequencies [VAFs] down to 0.001%). These targeted approaches usually entail the investigation of one to five genes (Kilgour et al., 2020).

Moreover, when a more extensive range of clinically significant mutations needs to be investigated, or if there is limited prior knowledge of mutations associated with the tumor, analyzing larger gene panels (spanning from 10 to hundreds of genes) or employing genome-wide approaches becomes essential. For such thorough panels, ctDNA analysis using ddPCR and RT-PCR is not feasible; instead, next-generation sequencing (NGS) approaches are required. Pioneering researchers first reported in 2012 that somatic mutations affecting several genes in cancer patients may be detected by combining cfDNA screening with NGS (Forshew et al., 2012). Additionally, the approach, known as Tagged-amplicon deep sequencing (TAm-Seq), facilitated the analysis of nearly 6,000 genomic bases with VAFs as low as 2%, significantly expanding the capability of ctDNA detection (Bohers et al., 2021).

16.2.2 MicroRNAs as promising biomarkers

16.2.2.1 Background

MicroRNAs (miRNAs) have emerged as promising biomarkers in the diagnosis of human diseases, particularly cancers. Different studies have highlighted the potential of miRNAs as dynamic and precise biomarkers for cancer detection, holding promise as early noninvasive diagnostic markers. These small RNA molecules are crucial in regulating gene expression and are intimately involved in various cellular pathways, including those implicated in carcinogenesis (Cho, 2012). Moreover, the intricate interplay between miRNAs and cancer pathogenesis highlights the complex regulatory networks underlying cancer development and progression, suggesting that miRNAs are the main genetic indicators of cancer (Bhat et al., 2019).

In cancer, certain miRNAs function as oncogenes, encouraging the growth and spread of tumors, while others act as tumor suppressors, preventing cancer development. Additionally, some miRNAs play crucial roles in modulating metastasis, the spread of cancer from its original site to other parts of the body (Cho, 2011). The ability of miRNAs to regulate gene expression at posttranscriptional levels makes them attractive candidates for diagnostic and therapeutic purposes. As our understanding of miRNA biology continues to advance, these molecules will likely play increasingly significant roles in cancer diagnosis and treatment strategies in the future (Cho, 2012).

It is suggested that miRNAs primarily exert their regulatory effects by inhibiting translation. Comparing gene expression profiles between cancerous and normal tissues shows deregulation of both miRNAs and mRNAs, suggesting miRNA changes may contribute to tumorigenesis. MiRNAs involved in cancer are called oncomirs. The dysregulation of miRNAs in cancer highlights their potential as diagnostic and therapeutic targets. Understanding specific miRNAs' roles in tumorigenesis could lead to novel biomarkers for early cancer detection. It is indicated that miRNAs are considered key genetic indicators of cancer, underscoring their importance in cancer development and progression (Bhat et al., 2019; Inoue & Inazawa, 2021; Shafat et al., 2022). A prime example is the patterns of miRNA used previously by Yoon et al. to predict a 5-year survival rate in early-stage oral squamous cell carcinoma (OSCC) patients (Yoon et al., 2020).

Over and above, Zhang et al. conveyed the overexpression of miR-155 in patients with nonmuscle invasive bladder cancer (NMIBC). This study demonstrated that this miRNA can effectively distinguish between NMIBC patients, those with cystitis, and healthy controls. With a sensitivity and specificity of 80.2% and 84.6%, respectively, it highlights its potential as a biomarker for diagnosing NMIBC (Zhang et al., 2016). Additionally, lower levels of miR-214 were found to be significantly related with longer recurrence-free survival time in patients with NMIBC (Kim et al., 2013).

16.2.2.2 Challenges

Despite the promising future of microRNA as a noninvasive biomarker, working with miRNAs presents several significant challenges that must be addressed for their successful application in cancer diagnosis and treatment. First, the low abundance of miRNA in the blood, which also contains complex matrices with potential interfering biomolecules, such as proteins (Salim et al., 2022). Additionally, low sensitivity and specificity could be produced by utilizing the single miRNA approach in some types of cancer including breast cancer. Subsequently, combining multiple miRNAs is an effective approach to enhance accuracy (Jang et al., 2020).

These complexities pose additional barriers to miRNA-based diagnostics' standardization and broader adoption. To address this challenge, several strategies are employed, such as incorporating an enrichment step before global expression profiling (Precazzini et al., 2021). Another approach is to utilize NGS, identifying a broader range of miRNAs with high sensitivity. Nevertheless, compared to other techniques, NGS is more expensive and time-consuming. Alternatively, MS excels in quantification, allowing for miRNA sequencing, quantitation, and evaluating posttranscriptional modifications (Salim et al., 2022).

16.2.3 DNA methylation

16.2.3.1 Background

DNA methylation alterations are closely associated with carcinogenesis and hold great promise as cancer biomarkers because of their stability, prevalence, and availability in body fluids (Ibrahim et al., 2023).

DNA methylation is one of the most important epigenetic mechanisms in the development, maintenance, and spread of cancer. Thus cancer epigenetics research has focused on identifying differentially methylated regions (DMRs) that consist of several consecutive methylated CpG strands. DMRs are mainly identified in gene promoter regions, within genes' bodies, and in intergenic regulatory regions (Aran et al., 2011; Bert et al., 2013; Jones & Baylin, 2002; Suzuki & Bird, 2008). Hypermethylation in cancer leads to genetic inactivation of DNA repair genes and tumor suppressor genes that affect the disease phenotype and enhance the mutagenesis rate. As a result, processes including cell cycle, apoptosis, cell division, DNA repair, and DNA replication are directly influenced due to their relation to these genes (Ding et al., 2019; Lakshminarasimhan & Liang, 2016; Suzuki & Bird, 2008). Therefore cancer cells can be differentiated from healthy tissues due to the cells unique methylation profile that support the identification of the DNA tissue of origin (Liu et al., 2020; Moss et al., 2018).

Due to the early occurrence of hypermethylation in cancer development, we can use DNA methylation as potential diagnostic biomarkers as across all cancer progression stages it remains methylated. Hence, it is useful in patients with metastatic cancer and carcinoma of unknown primary as tumor origin can be predicted (Draškovič & Hauptman, 2024).

Some evidence shows that epigenetics, particularly DNA methylation, has a quintessential role in regulating OSCC progression. Genomic instability was reported by previous studies as a result of methylation and genes dysregulation that is involved in the etiology of OSCC (Viet & Schmidt, 2008; Viet et al., 2007; Viet et al., 2021). Therefore employing epigenetic biomarkers demonstrated significant prognostic potential in predicting 5-year mortality. For example, Viet et al. (2021) studied early-stage (I/II) OSCC patients and used 12 genes' methylation patterns to calculate patients' mortality risk (Viet, et al., 2021).

Regarding detecting methylation, saliva, brush swabs, and circulating tumor cells (CTCs) have been used by other studies to collect OSCC cells noninvasively for diagnosis. Saliva has not shown to be effective because of the significant differences in methylation patterns between cancer tissues and saliva (Arantes et al., 2018). Notably, no significant differences were exhibited in DNA yield between brush swabs and tissue samples. Additionally, molecular risk at the time of diagnosis can be successfully calculated by methylation data of brush swabs (Viet et al., 2021).

Over and above, it is reported that cell-free circulating DNA (cfDNA) contains unique epigenetic markers, including DNA methylation, in specific GC-rich segments. The promoters and first exons of many genes typically have these fragments, forming CpG islands. Hence, developing highly accurate biomarkers could be achieved by analyzing DNA methylation in cfDNA. These biomarkers will contribute to the identification, diagnosis, forecasting of therapy response, and prognosis (Levenson, 2010).

A prominent example is using methylation patterns as biomarkers of cisplatin resistance. Cisplatin is used as the initial line of treatment for patients with advanced-stage head and neck squamous cell carcinoma, administered either as a single agent or in combination with radiotherapy (Pignon et al., 2009). Although reduced patient survival could happen due to significant resistance to cisplatin, it is suggested that DNA methylation plays a crucial role in cisplatin resistance. Hence, methylation patterns are used as biomarkers of cisplatin resistance after the identification of defined methylation patterns related to the sensitivity and resistance to cisplatin in tumors (Viet et al., 2014).

16.2.3.2 Detecting methylation

The bisulfite modification procedure is a technique that is used to detect methylation by converting the unmethylated cytosines to uracils, leaving methylated cytosines intact. The resulting alterations in DNA sequence are then identified through various methods (Frommer et al., 1992). The emergence of minimally invasive techniques, such as liquid biopsies, creates an optimal environment for the progress and application of these methylation-based biomarkers (Ibrahim et al., 2023).

16.2.3.3 Challenges to methylation analysis

The heterogeneity of clinical specimens complicates data analysis due to the presence of diverse components, each with unique methylation patterns, which can

change over time. Unlike methylation in homogeneous samples, that is relatively straightforward. Furthermore, the methylation levels are affected by the natural progression of cancer-introducing cells with varying degrees of neoplastic transformation (Levenson, 2010).

16.2.4 Circulating tumor cells

16.2.4.1 Background

It was recorded that CTCs possess substantial metastatic capabilities. They enter the blood circulation after arising from primary or metastatic tumors originating from the epithelium. Since CTCs provide a dynamic perspective for monitoring tumor progression in real-time, they are considered a crucial part of liquid biopsy (Lee & Kwak, 2020; Wang et al., 2020).

CTCs display a distinct array of characteristics, incorporating epithelial, mesenchymal, and hybrid phenotypes, making their role in the metastatic process intricate to unravel. The dynamic phenotypic transitions CTCs undergo while navigating the circulatory system have been explained by recent studies. These transitions can profoundly affect their role to extravasate and establish colonies in distant organs. However, CTCs' precise functions in metastasis are unclear due to their heterogeneous nature (Zhang et al., 2019).

CTCs face significant obstacles, including surviving in the bloodstream. Platelets play a crucial part in shielding CTCs from immune surveillance by surrounding these tumor cells to save them from destruction by natural killer cells. Therefore interactions between platelets and CTCs are pivotal to comprehending to deciphering the mechanisms that CTCs use in immune defense invasion (Schlesinger, 2018). Hence, it was noticed that using anticoagulants contributes to the reduction of metastasis (Capuozzo et al., 2023). In addition, CTCs have been used in different studies as metastatic disease early markers, resulting in linking their presence to reduced survival, locoregional recurrence, and treatment resistance (Tada et al., 2020).

16.2.4.2 Detection

Because of the dilution happening for the genetic material of CTCs during traditional high-throughput sequencing analysis of tumor tissue, that kind of analysis is effective. However, the continuous innovation and advancement in the sequencing technique, producing single-cell sequencing technology. As a result, this technology has made it possible to isolate and characterize CTCs, offering a promising essential role in the treatment of cancer (Lim et al., 2019; Orrapin et al., 2023).

Over and above, genomics analysis of individual CTCs enables the exploration of tumor heterogeneity and evolutionary dynamics. Monitoring CTC counts over time serves as a method to assess treatment responses. In certain cancers, CTCs can be collected to create patient-derived xenograft models, aiding in drug and

biomarker research. Ongoing efforts are focused on refining direct CTC cultures to enable real-time treatment testing, potentially enhancing clinical decision-making processes (Drapkin et al., 2018; Lu et al., 2020; Salu & Reindl, 2024).

16.2.5 Metabolites

By detecting specific metabolites associated with cancerous tissues, clinicians may be able to improve the accuracy of renal cell carcinoma (RCC) diagnosis and tailor treatment strategies based on individual metabolic profiles.

Research on RCC faces significant challenges due to the absence of reliable pre-operative diagnostic markers. Distinguishing clear cell RCC (ccRCC) from other subtypes of kidney cancer is particularly crucial for guiding treatment decisions and predicting patient outcomes. Therefore Urquhart's team discovered isovalerylglycine, and α-ketobutyrate that are two new kidney cancer biomarkers, through 2D-COSY spectra analysis (Urquhart et al., 2023). Isovalerylglycine is a metabolite generated during the breakdown (catabolism) of the amino acid leucine (Wishart et al., 2018). α-Ketobutyrate is produced through the hydrolysis of cystathionine to cysteine and α-ketobutyrate, catalyzed by γ-cystathioninase (Jung et al., 2013). Thus ccRCC can be distinguished from non-ccRCC and noncancer kidney.

16.3 Technologies using

In recent years, significant developments in cancer biomarker research have mirrored the rapid development of cutting-edge technologies. Particularly notable are the remarkable strides made in genomic and proteomic analytical methods. These sophisticated techniques have unveiled intricate signaling networks within cancer cells, shedding light on their role in disease initiation and progression. Furthermore, they have highlighted the profound impact that genetic and protein alterations exert on oncogenic signaling pathways. The tumors' heterogeneous nature underscores the complexity of these molecular alterations, emphasizing their combined influence on tumorigenesis and disease evolution (Dagogo-Jack & Shaw, 2018; Karczewski & Snyder, 2018).

Traditional techniques for biomarker identification face technological limitations, including enzyme-linked immunosorbent assay (ELISA) and PCR. These include the expensive reagents required for each assay and the relatively slow detection rate (Kumar et al., 2006). Furthermore, as manual procedures, they lack the capability for continuous patient monitoring during therapy. Cancer research is advancing through innovative molecular methods, enhancing our comprehension of the disease and uncovering potential new genomic and proteomic biomarkers. Addressing the challenges in cancer diagnosis necessitates multi-analyte analysis facilitated by lab-on-a-chip point-of-care (POC) devices (Ahn et al., 2004).

In the biomarker evaluation process, gene expression profiling and MS are used in preclinical screening to identify cancer markers. There is a need for noninvasive methods to detect biomarkers in tumor tissues or fluids. Consequently, clinical assays have been developed for various purposes: for instance, prostate-specific antigen (PSA) in prostate cancer screening, EGFR mutations in lung cancer diagnosis, hormone receptor status in breast cancer prognosis, and gene signatures for predicting treatment response to immunotherapy (Passaro et al., 2024).

Thus oncogenes and tumor-suppressor genes identifications have been improved by genome sequencing, accelerating cancer biomarkers' discovery. These biomarkers now serve as valuable tools for screening, diagnosing, prognosing, and predicting cancer outcomes. Initially grounded in empirical observations, early investigations into cancer biomarkers have evolved in parallel with advancements in testing technologies (Dakal et al., 2020; Passaro et al., 2024).

Tumor purity, which denotes the percentage of tumor cells within tumor tissue, has emerged as a critical factor influencing various aspects of cancer research and clinical outcomes. Recent studies have highlighted its significant impact on gene clustering, molecular taxonomy, coexpression networks, and assessments of tumor prognosis and the tumor microenvironment (Aran et al., 2015; Rhee et al., 2018).

Consequently, various computational approaches were improved for estimating purity. These methods utilize various types of genomic data, including transcriptome data, and copy number variation data. For instance, one prominent method employs the random forest (RF) algorithm, which is based on the data of DNA methylation (Capper et al., 2018).

Due to the limitations of available analytical methods that traditionally assess DNA, RNA, or proteomic composition separately, many studies investigate individual genetic factors, transcriptomic changes, and dysfunctional proteins as isolated risk and prognostic indicators. However, appearing NGS has publicized a transformative shift in this landscape. NGS has significantly enhanced the sensitivity of genomic techniques, enabling researchers to analyze vast quantities of genetic data rapidly and cost-effectively. Consequently, there has been a significant increase in the identification of genetic factors associated with cancer (Gonzaga-Jauregui et al., 2012; Kilpivaara & Aaltonen, 2013; Roberts et al., 2012).

Recently, a plethora of new diagnostic tools have been yielding, offering the advantage of delivering precise and timely information, enabling the quantification of cancer cells and metastases.

16.4 Trends and innovation in detecting biomarkers

16.4.1 Multi-model approach

Due to using a single biomarker, cancer diagnosis is challenging due to statistical limitations and lack of specificity. For example, uncertainties in diagnosing prostate

cancer emerge as a result of rising PSA levels in both prostate cancer and benign conditions. Research shows that assessing multiple biomarkers simultaneously provides more accurate and robust prognostic information. Evaluating 4–10 biomarkers improves diagnostic value, enhancing early cancer detection and disease progression assessment (Cheng et al., 2024).

As we progress, there's a notable shift from the conventional one-size-fits-all approach toward more targeted testing and treatment strategies. While molecular pathology initially transformed precision oncology, early companion diagnostic assays cleared by the FDA relied on simpler molecular methods, typically focusing on single genes of interest (Dietel et al., 2013; Malone et al., 2020). However, with the advancements in NGS, there is now a rising prevalence of multitarget companion diagnostic assays (Campbell, 2020; Malone et al., 2020).

Continued cost reductions are paving the way for the simultaneous profiling of thousands of genomic regions. This trend suggests that multitarget panels may soon be available at a comparable price point to testing five to ten targets individually (Colomer et al., 2020).

In addition, histopathology and radiology are essential in clinical decision-making for cancer management (Davidson et al., 2013; Pomerantz, 2020). Therefore histopathological evaluation, crucial for studying tissue architecture, remains the gold standard in cancer diagnosis. Advances in whole-slide imaging are increasingly replacing traditional histopathology methods with digital pathology (Rahman et al., 2020; Yu & Snyder, 2016).

OSCC biomarker research focuses on developing a multi-gene risk score to tailor patient treatments more precisely. Researchers have explored various approaches to achieve this, including analyzing differences in gene expression, gene amplification and deletion, DNA methylation, and microRNA (miRNA) profiles (Wong et al., 2024).

Recently, it became evident that finding stage- and subtype-specific biomarker panels is associated with more precise molecular signatures. A prime example is the study published in 2004 that identified a 102-gene signature by comparing gene expression changes between patients with and without neck metastasis. As a result, this 102-gene signature predicted neck metastasis with an accuracy of 86% (Roepman et al., 2005; Roepman et al., 2006).

16.4.2 Using AI in biomarker discovery

With the abundance and intricacy of data related to genes and epigenetics, relying solely on pairwise correlations is often insufficient for making accurate predictions. Therefore, analytical tools are crucial to uncover new relationships, formulate novel models, and generate accurate predictions (Libbrecht & Noble, 2015). To develop novel therapies and predictive models for drug response, artificial intelligence (AI) excels in integrating cancer biomarker and imaging data from global research labs and clinical institutions encompassing genomics, proteomics, metabolomics, oncology clinics, imaging, and epidemiology. This leverages its capability to

analyze vast amounts of information and identify complex patterns that can enhance treatment strategies and outcomes (Kehl et al., 2021; You et al., 2022).

ML techniques are increasingly utilized to find genetic variations connected to diseases using genome-wide association studies (GWAS), enabling researchers to identify associations between specific genetic markers and disease susceptibility or progression (Ozaki et al., 2002). A combination of ML and deep learning (DL) methods was documented to enhance GWAS analysis (Mieth et al., 2016). Due to the challenge in improving the regulatory variants' mapping in noncoding regions that were identified by GWAS, a deep learning-based approach has been developed by Arloth et al. (2020) to demonstrate SNPs which was statistically significant.

Machine learning (ML) algorithms can combine multiple biomarkers to achieve exceptional insights in diagnosing, predicting, and making decisions for new anticancer therapies. By analyzing complex datasets, ML improves the identification of patterns and correlations that traditional methods might overlook; thus, improving the precision and effectiveness of cancer treatments (Koh et al., 2022; Kong et al., 2022; Nguyen et al., 2022).

Over and above, the rapid evolution of NGS and other analytic technologies has resulted in a massive influx of omics data. Consequently, AI, particularly machine learning algorithms, has emerged as essential for managing and extracting meaningful insights from this vast dataset. By leveraging AI, researchers can comprehensively analyze whole genome, epigenome, transcriptome, proteome, and metabolome data together. This integrated approach enables the identification of complex biomarkers and molecular signatures that may better predict treatment responses (Asada et al., 2021; Gao et al., 2022).

Genomic and epigenetic data-driven research involves thorough exploration of genome-wide data to uncover novel properties, rather than merely validating existing models (Brown & Botstein, 1999). These methods include identifying associations between genotypes and phenotypes, discovering biomarkers for personalized medicine, and mapping genomic regions such as transcriptional enhancers that are involved in biochemical activities. ML is designed to autonomously identify patterns in data, contrasting with traditional algorithms that are constrained by biased assumptions or specialized knowledge. Hence, ML is exceptionally well-suited for data-driven science, particularly in the realms of genomics and epigenomics (Libbrecht & Noble, 2015).

The future of precision oncology hinges on integrating detailed omics data and harnessing AI's analytical capabilities to expand the scope of patients benefiting from tailored treatments beyond those identified by current targeted-gene panel methods (Asada et al., 2021).

16.4.2.1 Challenges

It must integrate different high-dimensional multimodal biomedical data in fusion methods to be effective such as quantitative features, images, and text (Acosta et al., 2022). Preparing raw data for machine learning is challenging because these

methods require data to be vectorized. In addition, multimodal representation introduces several complexities: different modalities capture unique and non-matching features. Furthermore, the confidence, noise levels, and information quality can differ across modalities and observations (Jain et al., 2021).

However, the effectiveness of ML hinges significantly on the representation of data and the extraction of individual variables or features. Epigenetic data and various modalities are recognized as interconnected events that potentially interact to influence patterns of gene activity (Asada et al., 2021).

Building on these hypotheses, Wang et al. employed a deterministic ML approach using stacked denoising autoencoders (SdAs) to predict the degree of DNA methylation in a certain genomic area. This model leveraged 3D genome topology data and DNA sequences derived from Hi-C experiments (Goldbeter & Koshland, 1981).

Experimentalists have contributed critical insights into the biochemical pathways that govern cell behavior. Ordinary Differential Equation (ODE) models have provided predictions on various aspects of these pathways. For instance, the stimulus–response relationship was initially thought to be linear, where higher concentrations of growth factors elicited greater responses. However, certain components within these pathways do not adhere to this linear behavior; instead, they exhibit an all-or-none response. ODE modeling has elucidated mechanisms underlying such ultrasensitivity. This phenomenon takes place when enzyme-functioning proteins reach saturation (Goldbeter & Koshland, 1981; Kim & Ferrell, 2007).

16.4.3 Biosensor

Understanding the existence, progression, and responsiveness to treatment of different cancer types is made possible by the identification and analysis of cancer biomarkers. As a result, it helps in early diagnosis, monitoring disease progression, and tailoring personalized treatment plans, improving overall patient outcomes (Hristova & Chan, 2019). Hence, there is a strong demand for biosensors—sensitive, dependable, and reasonably priced diagnostic instruments for cancer detection

Biosensors play a pivotal role in detecting and quantifying specific biological markers or analytes such as proteins, DNA, RNA, and cells. They achieve this by translating signals from biological molecule interactions into electrical signals, which can then be measured as digital outputs. Moreover, biosensor technology offers the advantage of delivering precise and timely information, enabling the quantification of cancer cells and metastases (Hasan et al., 2021). A prime example is Bead-based biosensors that have appeared as viable diagnostic platforms due to their versatility, high sensitivity, and ability to multiplex beads. These sensors utilize a wide range of cancer biomarkers, enhancing their potential for accurate and efficient cancer diagnostics (Cheng et al., 2024).

Subsequently, tremendous advancements have been made in the creation of bead-based biosensors (Son et al., 2023). A prominent example is microfluidic bead-based biosensors that resulted after integration with microfluidics and nanotechnology to

improve assay performance and facilitate precise handling of samples and reagents. They have been innovated to facilitate on-chip sample processing, including the isolation and enrichment of cells or viruses. The integration of microfluidic platforms not only reduces sample volumes but also enhances mixing and accelerates reaction kinetics. These improvements contribute significantly to boosting the accuracy and efficiency of biomarker detection processes (Cheng et al., 2024; Sher et al., 2021). A semiconductor sensor incorporated microfluidic chip that combines DNA strand labeling and bead-based immunoassay has been created for the purpose of identifying protein biomarkers (Lin & Peng, 2015).

Another example is the evolution of point-of-care testing (POCT) for cancer diagnostics which has transformed the landscape of cancer detection, offering more efficient options for patients. It aims to decentralize the testing and evaluation of cancer biomarkers, bringing them closer to patients. POCT diagnostics facilitates quicker clinical decisions due to the real-time results it provides, reducing the time and resources necessary for diagnosis (Cheng et al., 2024). Thanks to improvements in the fields of nanotechnology, microfluidics, and biotechnology that together have revolutionized the capabilities of POCT devices (Xie et al., 2022).

16.4.4 Third-generation technologies

Single-molecule real-time sequencing (SMRT sequencing) represents one of the prominent examples of third-generation sequencing (TGS) technologies that have recently emerged. This most recent round of sequencing techniques fundamentally differs from second-generation sequencing (SGS) approaches by directly interrogating single DNA molecules rather than clusters of DNA templates. As a result, TGS offers various benefits over SGS(NGS), including the elimination of amplification biases (Roberts et al., 2013; Schadt et al., 2010).

Single nucleotide variants are the most prevalent type of somatic variants and have attracted significant interest due to their role in cancer progression (Tate et al., 2019). For cancer applications, using long-read sequencing approaches can detect cancer-associated structural variants (SVs), including large insertions, duplications, and translocations of variable genomic sequences (Chen & He, 2021). Improving the validation and classification of germline SVs were observed with long-read sequencing, overcoming NGS limitations (Thibodeau et al., 2020). Additionally, it is evidenced that this technology is optimal to identify SVs linked to cancer at a low level after applying nanopore sequencing by Norris et al. (2016). Notably, nanopore sequencing has the ability to detect diluted SVs at dilutions as low as 1:100.

16.5 Conclusion

Recent developments in the identification of cancer biomarkers include the use of biosensors, AI (ML and DL), and multi-omics approaches. While new biomarkers

are being found on a daily basis, the most widely utilized ones for cancer diagnosis are circulating DND, cirulating tumor cells, DNA methylation, miRNA, and metabolites.

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The Potential of Cancer Biomarkers

From Discovery to Clinical Application

Edited by
Pranav Kumar Prabhakar, Professor, Department of Biotechnology, School of Engineering and Technology, Nagaland University, Meriema, Kohima, Nagaland, India; Department of Research Impact & Outcome, Research and Development Cell, Lovely Professional University, Phagwara, Punjab, India

The Potential of Cancer Biomarkers: From Discovery to Clinical Application addresses the challenges related to knowledge dissemination, interdisciplinary understanding, diagnostic complexity, quality assurance, patient care, research advancement, education, and professional development within the field of clinical biochemistry. It provides a holistic solution by offering comprehensive insights and practical guidance to a diverse audience, contributing to the enhancement of healthcare and biomedical research. Each chapter explores the multifaceted landscape of biomarker research, offering a wealth of insights and expertise in genomics, proteomics, metabolomics, and more. Cancer biomarkers, the molecular signatures of cancer, hold immense promise in revolutionizing how we diagnose, treat, and personalize therapies for cancer patients. In this curated reference, esteemed contributors explore the multifaceted landscape of biomarker research, offering a wealth of insights and expertise in genomics, proteomics, metabolomics, and more. This book is not just for researchers and academics. It is a valuable resource for medical professionals seeking to bridge the gap between cutting-edge research and patient care. Oncologists, pathologists, and clinicians will discover the tangible impact of biomarkers on early detection, prognosis, and personalized treatment strategies.

Key Features

- Presents the impact of biomarkers on early detection, prognosis, and personalized treatment strategies
- Includes ethical and regulatory considerations surrounding biomarker use, guiding policymakers and ethicists through the complex terrain of biomarker-driven healthcare decisions
- Highlights clinical relevance of cancer biomarkers by showcasing real-world case studies and examples of how biomarkers have been utilized to improve cancer diagnosis, personalize treatment plans, and monitor treatment responses

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