Marking the Cancers: Importance of Biomarkers

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ABSTRACT

A biomarker is a specific property that can be assessed as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention. Biomarkers are useful in the diagnosis of many diseases, including cancer. The United States Food and Drug Administration (FDA) and the National Institutes of Health (NIH) collaborated to identify biomarkers and their categories, which are made publicly available through a constantly updated online document called the “Biomarkers, Endpoints, and other Tools” (BEST) website. According to their clinical application, the FDA-NIH Biomarker Working Group has classified biomarkers into seven categories: susceptibility and risk, diagnostic, monitoring, prognostic, predictive, pharmacodynamic and treatment response, and safety biomarkers. Blood, urine, stool, and, less commonly, exhaled breath, saliva/buccal swabs, cerebrospinal fluid (CSF), sputum, and other body fluids can all be used to study cancer biomarkers. Biomarkers can be detected using a variety of approaches. Fluorescence in situhybridization (FISH), Immunohistochemistry (IHC), Polymerase Chain Reaction (PCR), Enzyme-linked Immunosorbent Assay (ELISA), Flow Cytometry, Microarrays, and Next Generation Sequencing (NGS) are a few examples. Cancer biomarkers have a wide range of therapeutic applications, with the ultimate goal of achieving precision medicine to optimize cancer prevention, screening, and treatment regimens. These applications include risk assessment, screening and early detection, accurate diagnosis, patient prognosis, therapy prediction, cancer surveillance and response monitoring.

Keywords—cancer biomarkers; susceptibility and risk biomarkers; diagnostic biomarkers; predictive biomarkers; detection methods

#  INTRODUCTION

A biomarker/biological marker is a characteristic that is measured as an indicator of risk and occurrence of disease, or patient outcome [1]. The term "biological marker" was introduced in the 1950s. In 1987, the U.S. National Academy of Sciences/National Research Council’s Committee on Biological Markers defined biological markers as “indicators signaling events in biological systems or samples” that could be classified into three categories: exposure, effect and susceptibility markers [2]. The term “biomarker”, refers to a broad subcategory of medical signs – that is, objective indications of medical state observed from outside the patient – which can be measured accurately and reproducibly [3]. The basic definition of a biomarker is simple: “A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention” [4]. In 1998, the [National Institutes of Health](https://en.wikipedia.org/wiki/National_Institutes_of_Health) Biomarkers Definitions Working Group defined a biomarker 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”[5]. According to the National Cancer Institute (NCI), a 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. A biomarker can also be defined as a group of variations, such as gene expression, proteomic, and metabolomic signatures. Biomarkers can be detected in the circulation (whole blood, serum, or plasma) or excretions or secretions (stool, urine, sputum, or nipple discharge), and thus easily analyzed non‐invasively and serially, or they can be tissue‐derived, and require either biopsy or specific imaging to be evaluated [6].

Biomarkers play an important role in the identification of various diseases including cancer. Cancer is defined as the abnormal proliferation of cells capable of infiltrating and spreading to other sections of the body. According to the World Health Organization, it is one of the main causes of death, with around 10 million fatalities projected globally by 2020. Biomarkers in oncology can be used for a variety of purposes, including determining an individual's risk of developing cancer, predicting the likelihood that a given therapy will work for a specific patient, and monitoring disease progression to determine if a therapy is working [7]. A list of various biomarkers in different cancers is mentioned below (Table 1).

**Table 1: Various Biomarkers in Different Cancers**

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| --- | --- |
| Cancer Type | Biomarkers |
| Breast | HER2, BRCA1, BRCA2, CA15-3, miR-155, Mucin-1, ErbB2, EGFR |
| Ovarian | CA 125, HE4, miR-126, miR-92, miR-93, Mesothelin |
| Bladder | hOGG1, BTA, COX-2, miR-126, miR-141-3p, IL-8, FDP, NMP22 |
| Brain | MGMT, p14arf, COX-2, miR-10b |
| Gastric | CEA, CA19-9, miR-29c, miR-148a |
| Lung | CEA, miR-106a-5p, miR-10b-5p, miR-141-3p, ALK, KRAS |
| Liver | Α-Fetoprotein, miR-100-5p, miR-122, HCCR-1 |
| Prostate | PSA, PCA3, GSTP1, miR-103a, miR-106a, miR-107, p63, Gleason |
| Melanoma | EGFR, HER3, ERK, NCOA3, miR-221 |

# TYPES OF BIOMARKERS

The U.S. Food and Drug Administration (FDA) and the National Institutes of Health (NIH) jointly defined biomarkers and their types, which are made publicly available through a continuously updated online document-the “Biomarkers, Endpoints, and other Tools” (BEST) resource. The FDA-NIH Biomarker Working Group has classified biomarkers into seven categories based on their clinical applications: susceptibility and risk, diagnostic, monitoring, prognostic, predictive, pharmacodynamic and treatment response, and safety (Figure 1) [8,9].

A. Susceptibility and risk biomarkers:

These biomarkers indicate the potential for developing a disease or medical condition in an individual who does not currently have a clinically apparent disease or medical condition. It is related to an increased, or in some situations, lower risk of getting a disease or medical condition in a person who does not have that disease or medical condition clinically. The primary role of susceptibility/risk biomarkers in clinical practice is to guide preventive interventions. This utility is influenced in part by the availability of therapies to reduce disease risk. A few examples of [susceptibility/risk biomarker](https://www.ncbi.nlm.nih.gov/books/n/biomarkers/glossary/def-item/glossary.susceptibility-risk-biomarker/)s are BReast CAncer gene 1/2 (BRCA1/2) mutation to evaluate the chance of developing breast and ovarian cancers, cytochrome P450 1A1 (CYP1A1) polymorphisms for the classification of patients at higher risk of gall bladder cancer and urinary concentration of tobacco-specific nitrosamines (TSNAs) for head and neck cancer [10,11,12]. These biomarkers could be used to assess whether lifestyle, nutritional, or other preventive treatments are necessary. Susceptibility/risk biomarkers may also be used to identify patients who require more intensive disease surveillance, such as more regular mammography to test for breast cancer [13].

B. Diagnostic biomarkers:

Medical practice requires accurate diagnosis of diseases and conditions. A diagnostic [biomarker](https://www.ncbi.nlm.nih.gov/books/n/biomarkers/glossary/def-item/glossary.biomarker/) is used to detect or confirm the presence of a disease or condition of interest or to identify individuals with a subtype of the disease. They are used to determine whether a patient has a particular medical condition for which treatment may be indicated or whether an individual should be enrolled in a clinical trial studying a particular disease. It is critical to characterize the predicted performance of a diagnostic biomarker test under the stipulated conditions of use. This entails paying attention to the population with the goal of diagnosing and how the test is applied to that population [14]. A few examples of diagnostic biomarkers include estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2) for breast cancer, cancer antigen 125 (CA 125) for ovarian cancer and prostate-specific antigen (PSA) for prostate cancer [15,16,17].

C. Monitoring biomarkers:

A monitoring biomarker is a [biomarker](https://www.ncbi.nlm.nih.gov/books/n/biomarkers/glossary/def-item/glossary.biomarker/) measured repeatedly for assessing the status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent. These biomarkers can be used to assess disease progression, which includes the occurrence of new disease effects, worsening of previously existing abnormalities, or a change in disease severity or specific abnormalities, as well as the response of a disease or condition to treatment, which can be positive or negative. Monitoring biomarkers during an intervention can be used for a variety of objectives, including evaluating how a drug is metabolized by a patient by monitoring drug concentration, detecting therapeutic effects or disease progression while on or post-therapy, and detecting toxicity. Monitoring biomarkers can be used to detect the existence of diseases or medical disorders, as well as the risk of developing them, at the individual or community level. Individuals being monitored may have no clinically obvious medical disorders or diseases, or they may have a medical condition or prior exposure that predisposes them to develop a new ailment or disease [18]. A few examples include monoclonal protein (M protein) to determine whether individuals with monoclonal gammopathy of undetermined significance (MGUS) are showing signs of progressing to other disorders, such as some types of blood cancer, prostate-specific antigen (PSA) for assessing disease status or burden in patients with prostate cancer and cancer antigen 125 (CA 125) to assess disease status or burden during and after treatment in patients with ovarian cancer [19,20,21].

D. Prognostic biomarkers:

Prognostic biomarkers are used to identify the likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest. It denotes a higher or lower chance of a future clinical occurrence, disease recurrence, or progression in a specific group. Prognostic biomarkers are assessed at a predetermined baseline, which may include a background treatment. Many well-known instances of prognostic biomarkers occur in clinical settings where a person has been diagnosed with an illness or condition and there is a desire to predict the likelihood of a future clinical occurrence [22]. Prognostic biomarkers are frequently employed in clinical trials as eligibility criteria to identify patients who are more likely to have clinical events or disease progression. According to the United States Food and Drug Administration (2012), prognostic biomarkers are commonly utilized as enrichment factors in drug development. Some examples of prognostic biomarkers include BReast CAncer genes 1 and 2 (BRCA1/2) mutations for the evaluation of women with breast cancer and for the assessment of the occurrence of a second breast cancer [23], chromosome 17p deletions and TP53 mutations for evaluating patients with chronic lymphocytic leukemia and to assess the likelihood of death [24,25], increasing prostate-specific antigen (PSA) and Gleason to evaluate patients with prostate cancer during follow-up and to assess the likeliness of cancer progression [26,27].

E. Predictive biomarkers:

Biomarkers used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent are defined as predictive biomarkers. They are used to identify individuals who are more prone to react to a specific medical product or environmental contaminant. The reaction could be a symptomatic benefit, improved survival, or an unfavorable effect. The term “predictive biomarker” refers to a wide range of interventions, including medications, biologics, medical devices or procedures, and behavioral or nutritional changes for the treatment or prevention of diseases or ailments. Predictive biomarkers are useful not just in clinical trials, but also in directing patient care decisions, such as evaluating who could benefit from a certain treatment or choosing between numerous interventions. In the latter case, proof indicating a biomarker predicts the comparative effectiveness of an intervention should be accompanied by a description of the alternative interventions being compared. Predictive biomarkers for intervention effects may include aspects of the individual's biological composition ("host characteristics") or characteristics of the disease process or other medical conditions [28]. A few examples include squamous differentiation in non-small cell lung cancer (NSCLC) to identify patients who should avoid treatment with pemetrexed because it is likely to result in worse survival or progression-free survival outcomes compared to treatment with other standard chemotherapies such as docetaxel or cisplatin in combination with gemcitabine, BReast CAncer genes 1 and 2 (BRCA1/2) mutations for evaluating women with platinum-sensitive ovarian cancer and to identify patients likely to respond to Poly (ADP-ribose) polymerase (PARP) inhibitors [29,30].

F. Response biomarkers:

It is a [biomarker](https://www.ncbi.nlm.nih.gov/books/n/biomarkers/glossary/def-item/glossary.biomarker/) used to show that a biological response, potentially beneficial or harmful, has occurred in an individual who has been exposed to a medical product or an environmental agent. They are classified into two types, pharmacodynamic [biomarker](https://www.ncbi.nlm.nih.gov/books/n/biomarkers/glossary/def-item/glossary.biomarker/) that indicates biologic activity of a medical product or environmental agent without necessarily drawing conclusions about efficacy or disease [outcome](https://www.ncbi.nlm.nih.gov/books/n/biomarkers/glossary/def-item/glossary.outcome/) or necessarily linking this activity to an established mechanism of action. A pharmacodynamic biomarker could be used to demonstrate proof-of-concept, assist in dose selection, or quantify a reaction to medical goods or environmental agents, including as a measure of possible risk. In some circumstances, such metrics may be secondary objectives in clinical studies and described in labeling [31]. Fluoroestradiol F-18 levels visualized by positron emission tomography (PET) to detect the response of estrogen receptor (ER) positive lesions to endocrine therapy in patients with recurrent or metastatic breast cancer, and phospho-AKT levels to measure inhibition of downstream phosphoinositide 3-kinase (PI3K) signaling as a response to anti-cancer PI3K inhibitors in paired tumor samples when evaluating target engagement of these drugs are some examples [32,33]. Surrogate endpoint biomarkers, on the other hand, are endpoints used in clinical trials as a substitute for a direct evaluation of how a patient feels, functions, or survives. Surrogate endpoints are supposed to predict clinical benefit or harm based on epidemiologic, therapeutic, pathophysiologic, or other scientific findings rather than measuring the clinical benefit of primary interest in and of itself. Depending on the amount of evidence, response biomarkers used to predict benefit in clinical trials may be considered candidate, reasonably likely, or validated surrogate endpoints. Response biomarkers can be employed in clinical care settings in addition to medical product development. The primary application of response biomarkers in clinical practice is to advise dose or administration.

G. Safety biomarkers:

Safety [biomarker](https://www.ncbi.nlm.nih.gov/books/n/biomarkers/glossary/def-item/glossary.biomarker/) is measured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect. The potential to identify or predict harmful medication or exposure effects is shared by all safety biomarkers. In some circumstances, toxicity is signaled by the discovery or change in a biomarker, allowing dose modification or treatment termination before toxicity becomes severe. In other circumstances, the safety biomarker may suggest that treatment is required. Furthermore, safety biomarkers can be utilized to identify patients for whom specific therapy should not be undertaken due to high safety hazards. A safety biomarker is the use of neutrophil count to evaluate patients on cytotoxic chemotherapy in order to alter the dose, identify the necessity to discontinue therapy, or explore the use of growth factors [34].

 

**Figure 1: Categories of Biomarkers based on Clinical Usage**

# SOURCES OF BIOMARKERS

A variety of sample sources can be used to investigate biomarkers for cancer, with the most commonly used being tumor tissue. Liquid biopsies, which are mostly non-invasive, are an alternative to tumor biopsies and the most commonly used specimen types for the analysis of cancer biomarkers that include blood, urine, stool, and, in less frequent cases, exhaled breath, saliva/buccal swabs, cerebrospinal fluid, sputum and other body fluids (Figure 2). These biomarkers are a great alternative to taking biopsies of certain organs for molecular analysis, which is why there is currently a focus on “liquid biopsies” [1].

A. Blood:

Cancer biomarkers can be released into the bloodstream as a result of active secretion or cellular leakage from tumor cells or supportive tissues in the tumor environment. These circulating biomarkers, which include proteins and autoantibodies, nucleic acids such as cell-free DNA and RNA, circulating tumor cells and microvesicles, can be utilized to evaluate tumor burden and metastatic potential while also providing insight into molecular changes in a tumor [35].

B. Urine:

Urine has been shown to be a reliable source of biomarkers in urinary tract cancers such as bladder, kidney, and prostate cancer [36]. Compared to blood biomarkers, which require the ability to relate peripheral markers to the intricate tumor microenvironment, urinary exosomes appear to be more focused as they are derived from relatively proximate cancer tissues and have a common embryonal lineage and are used to detect membrane proteins from tumors [37].

C. Stool:

A fecal examination is the most commonly used procedure for clinical examination and the most commonly used approach for census. It is simple, non-intrusive, and suitable for extensive population screening. Advancements in molecular biology have led to several attempts to identify novel fecal biomarkers for the early detection of colorectal cancer (CRC). The Fecal Occult Blood Test (FOBT), for example, is widely accepted as a non-invasive test in comparison with colonoscopy. Fecal biomarkers have the potential to improve screening acceptance, as they are non-invasive, easy, cost-effective, and safe to use as a screening and diagnostic technique for CRC screening and diagnosis [38]. The potential of DNA, RNA, proteins, microorganisms, and volatile chemical compounds in feces as biomarkers for CRC screening and early diagnosis has received a lot of interest. These biomarkers are predicted to become a new focus for the next generation of CRC screening and early diagnostic tests due to their simple sampling and minimal risk [39].

D. Breath:

Lung cancer is a major contributor to cancer prevalence and mortality worldwide, and the development of clinically relevant biomarkers to aid in the diagnosis of lung cancer at both the initial and subsequent stages is of paramount importance to the medical community. Despite the progress in treatment and the early detection of the malignancy, the majority of diagnoses are made at a later stage, when numerous genetic and epigenetic alterations have taken place. Exhaled Breath Condensate (EBC), a biological fluid, is a potential source of biomarkers that reflect the pathophysiology of lung cancer, containing molecules such as DNA, RNA, protein, metabolite, and volatile chemicals. The presence/absence of these molecules or their fluctuation in quantities are used as biomarkers [40]. EBC is a non-invasive resource for the evaluation of genetic markers and can are to assist in the diagnosis of illness, as well as in the evaluation of follow-up and/or therapy effectiveness [41].

E. Saliva:

The use of saliva for cancer diagnosis prior to the emergence of clinical, histological, and radiological indications is a potential method for establishing personalized treatment strategies [42]. Many recent studies have employed saliva, a biofluid that reflects the health of the body, to screen, diagnose, and follow breast cancer patients [43]. Saliva has several advantages, including ease of collection, little staff training, rapid sampling, hassle-free storage, ease of transportation, less susceptibility to clotting, and fewer dangers for health care providers [42,44]. Saliva is a possible non-invasive source of new biomarkers for cancer diagnosis and prognosis. For example, in a study, the meta-analysis of salivary biomarkers helped in the diagnosis of malignant non-oral tumors [45].

 

**Figure 2: Sources of Biomarkers**

# DETECTION OF BIOMARKERS

Various methods are used for the detection of cancer biomarkers. Some of them are listed below:

A. Fluorescence In SituHybridization (FISH):

FISH is a test that "maps" the genetic material in human cells, including individual genes or segments of genes. A FISH test helps in the discovery and diagnosis of cancer-related genetic alterations. It also provides extra information used to forecast a patient's fate and whether he or she will respond to chemotherapy medications [46,47]. For example, a FISH test is used to examine breast cancer tissue for the detection of multiple copies of HER2/neu gene. These cells develop more HER2 receptors, which receive signals that promote breast cancer cell proliferation. Blocking these receptors with trastuzumab (Herceptin) can be beneficial in treating patients with breast cancer who have multiple copies of the gene [48,49]. Additionally, testing urine cells for FISH is more accurate than a standard test that is used to identify abnormal cells and is intended for the diagnosis of bladder cancer. FISH can also detect the recurrence of bladder cancer up to six months earlier [50]. FISH can be used to identify chromosome abnormalities in certain types of leukemia, particularly those associated with more aggressive forms of Chronic Lymphocytic Leukemia (CLL), which may require immediate treatment [51,52].

B. Polymerase Chain Reaction (PCR):

The PCR assay has been demonstrated to be capable of detecting a single tumor marker-expressing cell in a population of up to 100 million lymphocytes, and has been used to identify tumor cells in approximately 18 solid tumor forms, the most widely studied being melanoma, breast and prostate carcinoma. PCR-based methods have been employed to identify cancer cells in biopsy specimens of solid tissue and lymph nodes, as well as bone marrow and peripheral blood, and also in other bodily fluids. Numerous studies have demonstrated a high degree of specificity and sensitivity for tumor marker detection and a strong correlation between the PCR results and the presence of metastatic disease. In patients with localized or metastatic cancer, PCR reveals tumor marker-expressing cells that would otherwise be undetectable by conventional methods [53]. In one study, for example, the expression of the mammaglobin biomarker was utilized to predict lymph node metastases in breast cancer patients using RT-PCR [54]. Another study found that the CR-LDR-qPCR assay can detect 30 methylated copies of each of three BrCa-specific CpG markers when mixed with an excess of unmethylated CpG markers (3000 copies each), which is a reasonable approximation of BrCa ctDNA overwhelmed with peripheral blood cell-free DNA (cfDNA) when isolated from patient plasma [55].

C. Next-Generation Sequencing (NGS):

Next-generation Sequencing (NGS) is a high-throughput method that efficiently identifies the sequences of millions to billions of DNA fragments. NGS has showed enormous promise not only in detecting early cancer biomarkers, but also in assisting drug discovery efforts and guiding therapy. NGS applications have expanded rapidly, allowing the creation of diagnostic and prognostic biomarkers for a wide range of disease domains [56], including cancer. NGS technologies are frequently employed in clinical research initiatives, such as the Cancer Genom Atlas project, to identify patterns of variation that can be used as biomarkers for cancer diagnosis [57]. For instance, NGS testing in patients with NSCLC has been demonstrated to be able to detect a low-frequency variant of the EGFR gene, the T490M mutation, which has been shown to be resistant to gefitinib and erlotinib therapy and can influence medical decisions [58].

D. Flow Cytometry:

Flow cytometry is becoming increasingly used in biomarker research. Its multiparametric character allows it to deliver extremely precise information on every single cell in a diverse population. Flow cytometry is employed in both preclinical and clinical settings to generate biomarker data that can be used to drive decisions about dose selection in clinical trials, treatment options for cancer patients, and even the suitability of individuals for transplants [59]. With the combination of the two biomarkers, flow cytometry found 0.01% dysplastic cells in a background of normal cervical epithelial cells [60]. In another study, the use of flow cytometry aided in distinguishing breast cancer indicators. Thus, flow cytometry, in conjunction with morphological analysis and IHC, can overcome specific limitations of each technology and offer trustworthy data in a more timely and efficient manner, leading to advances in breast cancer detection and prognosis [61].

E. Microarray:

Microarrays have become a widely used tool for the analysis of tens of thousands of gene expression levels simultaneously. This has enabled the study of a variety of disorders, including cancer, through the use of microarray data analysis [62]. These patterns can be used in the diagnosis or prognosis of a disease, characterize a particular stage of the disease, or identify and hypothesize the importance of particular genes in the progression of the disease [63]. For example, in a study, 44 genes were upregulated in a group of cancer patients with unknown primary characteristics (CUP), six ribosomal protein (RPS) genes were identified, two of which are well-known for their involvement in the Mdm2-p53 pathway. Additionally, several genes related to metastasis and apoptosis were identified, suggesting that CUP may possess a biological property [64]. Microarray studies on prostate cancer revealed interesting molecular markers such as AMACR, EZH2, TMPRSS2-ERG, miR-221 and miR-141 [65].

F. Immunohistochemistry (IHC):

IHC is more generally available and technically less difficult, has the ability to produce clinically meaningful results in a short period of time. It is less expensive than molecular platforms. Several IHC assays for predictive biomarkers have already been utilized in everyday pathology practice. The most common immunohistochemistry prognostic and therapeutic markers in breast cancer are estrogen receptor (ER), human epidermal growth factor receptor-2 (HER2), Ki-67, progesterone receptor (PR), and p53 [66]. Among the diagnostic IHC biomarkers, carbohydrate antigen 19-9 (CA19-9) has been the gold standard IHC biomarker authorized by the FDA for the diagnosis of pancreatic cancer [67].

G. Enzyme-Linked Immuno Sorbent Assay (ELISA):

ELISA has been widely utilized in regular clinical diagnostics and is still regarded as the gold standard for protein identification in physiological samples. Because of their higher sensitivity, signal amplification, ease of handling, potential for automation and combination with miniaturized analytical systems, low cost, and relative simplicity for mass production, ELISA-based immunoassays for cancer biomarker detection have recently piqued the interest of many researchers [68]. For example, Nw-hydroxy L-Arginine (NOHA) was identified as a blood-derived biomarker used to differentiate breast cancer tumors classified as ER- or ER+ based on their disease burden, progression rate, and molecular profile. A novel ELISA-based assay utilizing specialized monoclonal antibodies (mAb) specifically designed for NOHA was found to be an effective tool for predicting ER-breast cancer and monitoring disease progression without the need for costly analytical equipment (LC-MS), large laboratory space, or technical training [69].

# CLINICAL APPLICATIONS

Cancer biomarkers have a wide range of therapeutic applications, including risk assessment, screening, early detection, diagnosis, prognosis, response to therapy, cancer surveillance, and monitoring response [1]. These biomarkers can be used to assess patients in a variety of clinical settings, such as to estimate risk of disease, screen for primary cancers, differentiate benign from malignant, or to predict the prognosis of patients with cancer. Additionally, they can be used to monitor the status of patients with cancer, either for the purpose of detecting recurrence or to determine the response or progression of therapy [6]. The ultimate goal of biomarkers is to achieve precision medicine, which can help to optimize cancer prevention and detection, as well as treatment regimens.

# CONCLUSION

Cancer cells undergo several modifications, and these changes have been utilized as cancer biomarkers for decades, mostly in tumor tissue. Recent research on cancer biomarkers has aided in the development of new DNA, RNA, and protein-based cancer biomarkers that can be identified in easily accessible body fluids. Biomarkers play an important role in the diagnosis and treatment of nearly every cancer patient. Prior to receiving regulatory clearance, new drugs must pass stringent scrutiny and be evaluated in properly designed, randomized clinical trials. Unfortunately, despite the fact that biomarkers can have a significant impact on patient outcomes, such regulations do not exist. Clinical, translational and laboratory researchers must therefore be cognizant of the difficulties associated with the development of suitable biomarkers to enable the transfer of clinically relevant biomarkers to the clinical setting, thus avoiding the introduction of non-validated biomarkers that may be ineffective or even detrimental to patient care.

##### REFERENCES

1. Sarhadi, Virinder Kaur, and Gemma Armengol. “Molecular Biomarkers in Cancer.” *Biomolecules*, vol. 12, no. 8, July 2022, p. 1021. *PubMed Central*, https://doi.org/10.3390/biom12081021.
2. Slikker, William. “Biomarkers and Their Impact on Precision Medicine.” *Experimental Biology and Medicine*, vol. 243, no. 3, Feb. 2018, pp. 211–12. *PubMed Central*, https://doi.org/10.1177/1535370217733426.
3. Strimbu, Kyle, and Jorge A. Tavel. “What Are Biomarkers?” *Current Opinion in HIV and AIDS*, vol. 5, no. 6, Nov. 2010, pp. 463–66. *PubMed Central*, https://doi.org/10.1097/COH.0b013e32833ed177.
4. Califf, Robert M. “Biomarker Definitions and Their Applications.” *Experimental Biology and Medicine*, vol. 243, no. 3, Feb. 2018, pp. 213–21. *PubMed Central*, https://doi.org/10.1177/1535370217750088.
5. Biomarkers Definitions Working Group. “Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework.” *Clinical Pharmacology and Therapeutics*, vol. 69, no. 3, Mar. 2001, pp. 89–95. *PubMed*, https://doi.org/10.1067/mcp.2001.113989.
6. Henry, N. Lynn, and Daniel F. Hayes. “Cancer Biomarkers.” *Molecular Oncology*, vol. 6, no. 2, Apr. 2012, pp. 140–46. *PubMed Central*, https://doi.org/10.1016/j.molonc.2012.01.010.
7. Kimmons, Lany. “How Are Biomarkers Used to Treat Cancer?” *MD Anderson Cancer Center*, https://www.mdanderson.org/cancerwise/how-are-biomarkers-used-in-cancer-treatment.h00-159460056.html. Accessed 4 Sept. 2023.
8. Shah, Ankeet, et al. “Classification of Molecular Biomarkers.” *Société Internationale d’Urologie Journal*, vol. 1, no. 1, 1, Oct. 2020, pp. 8–15.
9. FDA-NIH Biomarker Working Group. *BEST (Biomarkers, EndpointS, and Other Tools) Resource*. Food and Drug Administration (US), 2016. *PubMed*, http://www.ncbi.nlm.nih.gov/books/NBK326791/.
10. Petrucelli, Nancie, et al. “BRCA1- and BRCA2-Associated Hereditary Breast and Ovarian Cancer.” *GeneReviews®*, edited by Margaret P. Adam et al., University of Washington, Seattle, 1993. *PubMed*, http://www.ncbi.nlm.nih.gov/books/NBK1247/.
11. García, Patricia, et al. “Current and New Biomarkers for Early Detection, Prognostic Stratification, and Management of Gallbladder Cancer Patients.” *Cancers*, vol. 12, no. 12, Dec. 2020, p. 3670. *PubMed*, https://doi.org/10.3390/cancers12123670.
12. Khariwala, Samir S., et al. “Elevated Levels of 1-Hydroxypyrene and N′-Nitrosonornicotine in Smokers with Head and Neck Cancer: A Matched Control Study.” *Head & Neck*, vol. 35, no. 8, Aug. 2013, pp. 1096–100. *PubMed Central*, https://doi.org/10.1002/hed.23085.
13. “Susceptibility/Risk Biomarker.” *BEST (Biomarkers, EndpointS, and Other Tools) Resource [Internet]*, Food and Drug Administration (US), 2020. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK402288/.
14. Group, FDA-NIH Biomarker Working. “Diagnostic Biomarker.” *BEST (Biomarkers, EndpointS, and Other Tools) Resource [Internet]*, Food and Drug Administration (US), 2020. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK402285/.
15. Gamble, Paul, et al. “Determining Breast Cancer Biomarker Status and Associated Morphological Features Using Deep Learning.” *Communications Medicine*, vol. 1, no. 1, 1, July 2021, pp. 1–12. *www.nature.com*, https://doi.org/10.1038/s43856-021-00013-3.
16. Dochez, Vincent, et al. “Biomarkers and Algorithms for Diagnosis of Ovarian Cancer: CA125, HE4, RMI and ROMA, a Review.” *Journal of Ovarian Research*, vol. 12, no. 1, Mar. 2019, p. 28. *BioMed Central*, <https://doi.org/10.1186/s13048-019-0503-7>.
17. Tkac, Jan, et al. “Prostate-Specific Antigen Glycoprofiling as Diagnostic and Prognostic Biomarker of Prostate Cancer.” *Interface Focus*, vol. 9, no. 2, Feb. 2019, p. 20180077. *royalsocietypublishing.org (Atypon)*, https://doi.org/10.1098/rsfs.2018.0077.
18. “Monitoring Biomarker.” *BEST (Biomarkers, EndpointS, and Other Tools) Resource [Internet]*, Food and Drug Administration (US), 2021. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK402282/.
19. Kyle, Robert A., et al. “A Long-Term Study of Prognosis in Monoclonal Gammopathy of Undetermined Significance.” *The New England Journal of Medicine*, vol. 346, no. 8, Feb. 2002, pp. 564–69. *PubMed*, https://doi.org/10.1056/NEJMoa01133202.
20. Sandler, Howard M., and Mario A. Eisenberger. “Assessing and Treating Patients with Increasing Prostate Specific Antigen Following Radical Prostatectomy.” *The Journal of Urology*, vol. 178, no. 3 Pt 2, Sept. 2007, pp. S20-24. *PubMed*, https://doi.org/10.1016/j.juro.2007.04.034.
21. Gundogdu, Fatih, et al. “The Role of Serum CA-125 Levels and CA-125 Tissue Expression Positivity in the Prediction of the Recurrence of Stage III and IV Epithelial Ovarian Tumors (CA-125 Levels and Tissue CA-125 in Ovarian Tumors).” *Archives of Gynecology and Obstetrics*, vol. 283, no. 6, June 2011, pp. 1397–402. *PubMed*, https://doi.org/10.1007/s00404-010-1589-8.
22. “Prognostic Biomarker.” *BEST (Biomarkers, EndpointS, and Other Tools) Resource [Internet]*, Food and Drug Administration (US), 2016. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK402289/.
23. Basu, N. N., et al. “Risk of Contralateral Breast Cancer in BRCA1 and BRCA2 Mutation Carriers: A 30-Year Semi-Prospective Analysis.” *Familial Cancer*, vol. 14, no. 4, Dec. 2015, pp. 531–38. *PubMed*, https://doi.org/10.1007/s10689-015-9825-9.
24. Gonzalez, David, et al. “Mutational Status of the TP53 Gene as a Predictor of Response and Survival in Patients with Chronic Lymphocytic Leukemia: Results from the LRF CLL4 Trial.” *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, vol. 29, no. 16, June 2011, pp. 2223–29. *PubMed*, https://doi.org/10.1200/JCO.2010.32.0838.
25. Shanafelt, Tait D., et al. “Prospective Evaluation of Clonal Evolution during Long-Term Follow-up of Patients with Untreated Early-Stage Chronic Lymphocytic Leukemia.” *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, vol. 24, no. 28, Oct. 2006, pp. 4634–41. *PubMed*, https://doi.org/10.1200/JCO.2006.06.9492.
26. Roberts, S. G., et al. “PSA Doubling Time as a Predictor of Clinical Progression after Biochemical Failure Following Radical Prostatectomy for Prostate Cancer.” *Mayo Clinic Proceedings*, vol. 76, no. 6, June 2001, pp. 576–81. *PubMed*, https://doi.org/10.4065/76.6.576.
27. Epstein, Jonathan I., et al. “The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System.” *The American Journal of Surgical Pathology*, vol. 40, no. 2, Feb. 2016, pp. 244–52. *PubMed*, https://doi.org/10.1097/PAS.0000000000000530.
28. “Predictive Biomarker.” *BEST (Biomarkers, EndpointS, and Other Tools) Resource [Internet]*, Food and Drug Administration (US), 2016. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK402283/.
29. Scagliotti, Giorgio, et al. “The Differential Efficacy of Pemetrexed According to NSCLC Histology: A Review of Two Phase III Studies.” *The Oncologist*, vol. 14, no. 3, Mar. 2009, pp. 253–63. *PubMed*, https://doi.org/10.1634/theoncologist.2008-0232.
30. Ledermann, Jonathan, et al. “Olaparib Maintenance Therapy in Platinum-Sensitive Relapsed Ovarian Cancer.” *The New England Journal of Medicine*, vol. 366, no. 15, Apr. 2012, pp. 1382–92. *PubMed*, https://doi.org/10.1056/NEJMoa1105535.
31. “Response Biomarker.” *BEST (Biomarkers, EndpointS, and Other Tools) Resource [Internet]*, Food and Drug Administration (US), 2021. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK402286/.
32. Liao, Geraldine J., et al. “18F-Fluoroestradiol PET: Current Status and Potential Future Clinical Applications.” *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, vol. 57, no. 8, Aug. 2016, pp. 1269–75. *PubMed*, https://doi.org/10.2967/jnumed.116.175596.
33. Morschhauser, Franck, et al. “On-Target Pharmacodynamic Activity of the PI3K Inhibitor Copanlisib in Paired Biopsies from Patients with Malignant Lymphoma and Advanced Solid Tumors.” *Molecular Cancer Therapeutics*, vol. 19, no. 2, Feb. 2020, pp. 468–78. *PubMed*, https://doi.org/10.1158/1535-7163.MCT-19-0466.
34. “Safety Biomarker.” *BEST (Biomarkers, EndpointS, and Other Tools) Resource [Internet]*, Food and Drug Administration (US), 2016. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK402287/.
35. Marrugo-Ramírez, José, et al. “Blood-Based Cancer Biomarkers in Liquid Biopsy: A Promising Non-Invasive Alternative to Tissue Biopsy.” *International Journal of Molecular Sciences*, vol. 19, no. 10, Sept. 2018, p. 2877. *PubMed Central*, https://doi.org/10.3390/ijms19102877.
36. Pisitkun, Trairak, et al. “Discovery of Urinary Biomarkers.” *Molecular & Cellular Proteomics: MCP*, vol. 5, no. 10, Oct. 2006, pp. 1760–71. *PubMed*, https://doi.org/10.1074/mcp.R600004-MCP200.
37. Panfoli, Isabella. “Cancer Exosomes in Urine: A Promising Biomarker Source.” *Translational Cancer Research*, vol. 6, no. Suppl 8, Oct. 2017. *tcr.amegroups.org*, https://doi.org/10.21037/tcr.2017.10.17.
38. Bresalier, Robert S., et al. “Biomarkers for Early Detection of Colorectal Cancer: The Early Detection Research Network, a Framework for Clinical Translation.” *Cancer Epidemiology, Biomarkers & Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, vol. 29, no. 12, Dec. 2020, pp. 2431–40. *PubMed Central*, <https://doi.org/10.1158/1055-9965.EPI-20-0234>.
39. Ding, Qian, et al. “Fecal Biomarkers: Non-Invasive Diagnosis of Colorectal Cancer.” *Frontiers in Oncology*, vol. 12, Sept. 2022, p. 971930. *PubMed Central*, https://doi.org/10.3389/fonc.2022.971930.
40. Campanella, Annalisa, et al. “Exhaled Breath Condensate Biomarkers for Lung Cancer.” *Journal of Breath Research*, vol. 13, no. 4, Aug. 2019, p. 044002. *PubMed*, https://doi.org/10.1088/1752-7163/ab2f9f.
41. Youssef, Omar, et al. “Exhaled Breath Condensate as a Source of Biomarkers for Lung Carcinomas. A Focus on Genetic and Epigenetic Markers-A Mini-Review.” *Genes, Chromosomes & Cancer*, vol. 55, no. 12, Dec. 2016, pp. 905–14. *PubMed*, https://doi.org/10.1002/gcc.22399.
42. Kaczor-Urbanowicz, Karolina Elżbieta, et al. “Clinical Validity of Saliva and Novel Technology for Cancer Detection.” *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, vol. 1872, no. 1, Aug. 2019, pp. 49–59. *ScienceDirect*, https://doi.org/10.1016/j.bbcan.2019.05.007.
43. Koopaie, Maryam, Fatemeh Abedinejad, et al. “Salivary MiRNA-21 Expression as a Potential Non-Invasive Diagnostic Biomarker in Breast Cancer.” *Gene Reports*, vol. 25, Dec. 2021, p. 101317. *ScienceDirect*, https://doi.org/10.1016/j.genrep.2021.101317.
44. Koopaie, Maryam, Sajad Kolahdooz, et al. “Salivary Biomarkers in Breast Cancer Diagnosis: A Systematic Review and Diagnostic Meta-Analysis.” *Cancer Medicine*, vol. 11, no. 13, 2022, pp. 2644–61. *Wiley Online Library*, <https://doi.org/10.1002/cam4.4640>.
45. Rapado-González, Óscar, et al. “Salivary Biomarkers for Cancer Diagnosis: A Meta-Analysis.” *Annals of Medicine*, vol. 52, no. 3–4, 2020, pp. 131–44. *PubMed*, https://doi.org/10.1080/07853890.2020.1730431.
46. Ansorge, Rick. “FISH Test for Cancer.” *WebMD*, https://www.webmd.com/cancer/fish-cancer-test. Accessed 4 Sept. 2023.
47. Cui, Chenghua, et al. “Fluorescence In Situ Hybridization: Cell-Based Genetic Diagnostic and Research Applications.” *Frontiers in Cell and Developmental Biology*, vol. 4, Sept. 2016, p. 89. *PubMed Central*, <https://doi.org/10.3389/fcell.2016.00089>.
48. Gutierrez, Carolina, and Rachel Schiff. “HER 2: Biology, Detection, and Clinical Implications.” *Archives of Pathology & Laboratory Medicine*, vol. 135, no. 1, Jan. 2011, pp. 55–62. *PubMed Central*, <https://doi.org/10.1043/2010-0454-RAR.1>.
49. Iqbal, Nida, and Naveed Iqbal. “Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications.” *Molecular Biology International*, vol. 2014, 2014, p. 852748. *PubMed Central*, <https://doi.org/10.1155/2014/852748>.
50. Caraway, Nancy P., et al. “Fluorescence in Situ Hybridization for Detecting Urothelial Carcinoma: A Clinicopathological Study.” *Cancer Cytopathology*, vol. 118, no. 5, Oct. 2010, pp. 259–68. *PubMed Central*, <https://doi.org/10.1002/cncy.20099>.
51. Reddy, K. S. “Chronic Lymphocytic Leukaemia Profiled for Prognosis Using a Fluorescence in Situ Hybridisation Panel.” *British Journal of Haematology*, vol. 132, no. 6, Mar. 2006, pp. 705–22. *PubMed*, <https://doi.org/10.1111/j.1365-2141.2005.05919.x>.
52. Mukkamalla, Shiva Kumar R., et al. “Chronic Lymphocytic Leukemia.” *StatPearls*, StatPearls Publishing, 2023. *PubMed*, <http://www.ncbi.nlm.nih.gov/books/NBK470433/>.
53. Raj, G. V., et al. “Utilization of Polymerase Chain Reaction Technology in the Detection of Solid Tumors.” *Cancer*, vol. 82, no. 8, Apr. 1998, pp. 1419–42. *PubMed*, [https://doi.org/10.1002/(sici)1097-0142(19980415)82:8<1419::aid-cncr1>3.0.co;2-4](https://doi.org/10.1002/%28sici%291097-0142%2819980415%2982%3A8%3C1419%3A%3Aaid-cncr1%3E3.0.co;2-4).
54. Monsalve-Lancheros, Ana, et al. “Detection of Mammagloblin by RT-PCR as a Biomarker for Lymph Node Metastasis in Breast Cancer Patients: A Systematic Review and Meta-Analysis.” *PLoS ONE*, vol. 14, no. 5, May 2019, p. e0216989. *PubMed Central*, <https://doi.org/10.1371/journal.pone.0216989>.
55. Bacolod, Manny D., et al. “Prediction of Blood-Based Biomarkers and Subsequent Design of Bisulfite PCR-LDR-QPCR Assay for Breast Cancer Detection.” *BMC Cancer*, vol. 20, no. 1, Jan. 2020, p. 85. *BioMed Central*, <https://doi.org/10.1186/s12885-020-6574-4>.
56. Vuksanaj, Kathy. “Sequencing Assays for Biomarker Discovery and Pharmacodynamics in Oncology Studies.” *GEN - Genetic Engineering and Biotechnology News*, 11 Oct. 2022, <https://www.genengnews.com/resources/sequencing-assays-for-biomarker-discovery-and-pharmacodynamics-in-oncology-studies/>.
57. Tomczak, Katarzyna, et al. “The Cancer Genome Atlas (TCGA): An Immeasurable Source of Knowledge.” *Contemporary Oncology*, vol. 19, no. 1A, 2015, pp. A68–77. *PubMed Central*, <https://doi.org/10.5114/wo.2014.47136>.
58. Kobayashi, Susumu, et al. “EGFR Mutation and Resistance of Non-Small-Cell Lung Cancer to Gefitinib.” *The New England Journal of Medicine*, vol. 352, no. 8, Feb. 2005, pp. 786–92. *PubMed*, <https://doi.org/10.1056/NEJMoa044238>.
59. Barnard, Ruth M. “Flow Cytometry: A Flexible Tool for Biomarker Research.” *Bioanalysis*, vol. 4, no. 20, Oct. 2012, pp. 2471–83. *PubMed*, <https://doi.org/10.4155/bio.12.225>.
60. Ling, Jian, et al. “Application of Flow Cytometry for Biomarker-Based Cervical Cancer Cells Detection.” *Diagnostic Cytopathology*, vol. 36, Feb. 2008, pp. 76–84. *ResearchGate*, <https://doi.org/10.1002/dc.20763>.
61. Wopereis, Sandro, et al. “Evaluation of ER, PR and HER2 Markers by Flow Cytometry for Breast Cancer Diagnosis and Prognosis.” *Clinica Chimica Acta; International Journal of Clinical Chemistry*, vol. 523, Dec. 2021, pp. 504–12. *PubMed*, <https://doi.org/10.1016/j.cca.2021.11.005>.
62. Riker, Adam I., et al. “The Gene Expression Profiles of Primary and Metastatic Melanoma Yields a Transition Point of Tumor Progression and Metastasis.” *BMC Medical Genomics*, vol. 1, Apr. 2008, p. 13. *PubMed Central*, <https://doi.org/10.1186/1755-8794-1-13>.
63. Sánchez-Peña, Matilde L., et al. “Identification of Potential Biomarkers from Microarray Experiments Using Multiple Criteria Optimization.” *Cancer Medicine*, vol. 2, no. 2, Apr. 2013, pp. 253–65. *PubMed Central*, <https://doi.org/10.1002/cam4.69>.
64. Kurahashi, Issei, et al. “A Microarray-Based Gene Expression Analysis to Identify Diagnostic Biomarkers for Unknown Primary Cancer.” *PLOS ONE*, vol. 8, no. 5, May 2013, p. e63249. *PLoS Journals*, <https://doi.org/10.1371/journal.pone.0063249>.
65. Sørensen, Karina Dalsgaard, and Torben Falck Ørntoft. “Discovery of Prostate Cancer Biomarkers by Microarray Gene Expression Profiling.” *Expert Review of Molecular Diagnostics*, vol. 10, no. 1, Jan. 2010, pp. 49–64. *Taylor and Francis+NEJM*, <https://doi.org/10.1586/erm.09.74>.
66. Zaha, Dana Carmen. “Significance of Immunohistochemistry in Breast Cancer.” *World Journal of Clinical Oncology*, vol. 5, no. 3, Aug. 2014, pp. 382–92. *PubMed Central*, <https://doi.org/10.5306/wjco.v5.i3.382>.
67. Igbinigie, Eseosaserea, et al. “Dkk1 Involvement and Its Potential as a Biomarker in Pancreatic Ductal Adenocarcinoma.” *Clinica Chimica Acta; International Journal of Clinical Chemistry*, vol. 488, Jan. 2019, pp. 226–34. *PubMed*, <https://doi.org/10.1016/j.cca.2018.11.023>.
68. Arya, Sunil K., and Pedro Estrela. “Recent Advances in Enhancement Strategies for Electrochemical ELISA-Based Immunoassays for Cancer Biomarker Detection.” *Sensors*, vol. 18, no. 7, 7, July 2018, p. 2010. *www.mdpi.com*, <https://doi.org/10.3390/s18072010>.
69. Mohan, Srinidi, et al. “Competitive ELISA Method for Novel Estrogen-Negative Breast Cancer Biomarker Quantitation.” *Journal of Immunological Methods*, vol. 474, Nov. 2019, p. 112671. *PubMed*, https://doi.org/10.1016/j.jim.2019.112671.