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 signalling 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 associated with an elevated risk, or in certain cases a decreased chance, of developing a disease or medical condition in a person who does not already clinically have that disease or medical condition. Susceptibility/risk biomarkers' main use in clinical practice is to direct preventive measures. The availability of treatments to lower disease risk has an impact on this utility to some extent. Examples of susceptibility/risk biomarkers include 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 and BRreast CAncer gene 1/2 (BRCA1/2) mutation to assess the likelihood of developing breast and ovarian cancers [10,11,12]. These biomarkers could be used to determine the need for dietary, lifestyle, or other preventive therapies. Patients who require more intense disease surveillance, such as more frequent mammograms to check for breast cancer, may also be identified using susceptibility/risk biomarkers [13].

B. Diagnostic biomarkers:

The proper diagnosis of illnesses and ailments is essential to good medical practice. When an illness or condition is present, a diagnostic biomarker is utilized to either confirm it or to identify individuals who have a particular 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 crucial to describe the anticipated performance of a diagnostic biomarker test under the predetermined usage circumstances. This necessitates paying attention to the demographic being diagnosed and how the test is being used on 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 that is regularly examined to determine the progression of a disease or other medical condition, as well as to show exposure to (or the impact of) a drug or other environmental factor. These biomarkers can be used to monitor disease progression, which includes the appearance of new disease effects, a worsening of already present abnormalities, a change in the severity of the disease, or a change in a particular abnormality, as well as the disease or condition's response to treatment, which may be favourable or unfavourable. Monitoring biomarkers during an intervention can be utilized for various objectives, including assessing 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 not exhibit any clinically evident medical illnesses or diseases, or they may have a health issue or history of exposure that makes them more likely to experience the onset of a new disorder or disease [18]. For instance, prostate-specific antigen (PSA) is used to assess the disease status or burden in patients with prostate cancer, while cancer antigen 125 (CA 125) is used to assess the disease status or burden in patients with ovarian cancer both during and after treatment and monoclonal protein (M protein) to assess whether people with monoclonal gammopathy of undetermined significance (MGUS) are displaying symptoms of progression to other conditions [19,20,21].

D. Prognostic biomarkers:

The chance of a clinical occurrence, illness recurrence, or disease progression in patients with the disease or medical condition of interest is determined by prognostic biomarkers. It indicates a greater or lesser likelihood of future clinical incidence, disease development, or recurrence in a certain group. Prognostic biomarkers are evaluated at a pre-established baseline, which may also contain a background therapy. Prognostic biomarkers are frequently used in clinical settings where a patient has been diagnosed with a disease or condition and it is desired to determine the chance of a future clinical occurrence [22]. In clinical trials, prognostic indicators are routinely used as eligibility requirements to identify patients who are more likely to experience 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 the evaluation of patients with chronic lymphocytic leukemia and to determine the likelihood of death [24,25], increasing prostate-specific antigen (PSA) and Gleason to evaluate patients with prostate cancer during check-up and to evaluate the likeliness of development of cancer [26,27].

E. Predictive biomarkers:

Predictive biomarkers are those that are used to identify people who are more likely than similar people without the biomarker to suffer a positive or negative reaction to exposure to a drug or an environmental contaminant. They are employed to pinpoint those who are more likely to respond adversely to a given medicinal product or environmental contaminant. The response could have a positive impact on symptoms, lead to increased survival, or have a negative outcome. The phrase "predictive biomarker" covers a broad range of therapies for the treatment or prevention of diseases or afflictions, including drugs, biologics, medical devices or procedures, as well as behavioural or dietary changes. In addition to clinical trials, predictive biomarkers are helpful when making decisions about patient care, such as determining who would benefit from a certain treatment or selecting amongst several approaches. In the latter scenario, evidence demonstrating that a biomarker predicts the relative efficacy of an intervention should be supported by a description of the alternative interventions being contrasted. The biological makeup of the person (their "host characteristics"), the characteristics of the illness process, or other medical disorders may all serve as predictive biomarkers for the impact of interventions [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 with conventional chemotherapies like cisplatin or docetaxel in combined 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 that is used to demonstrate that a biological reaction, which may be advantageous or harmful, has taken place in a person who has been exposed to a drug or an environmental pollutant. They can be divided into two categories: pharmacodynamic biomarkers, which show the biological activity of a drug or environmental agent without necessarily inferring anything about the efficacy or course of a disease or connecting this activity to a known mechanism of action. A pharmacodynamic biomarker could be employed as a proof-of-concept, to help choose the appropriate dose, or to quantify a response to drugs or environmental irritants, including as a gauge of potential danger. Such measurements may occasionally serve as ancillary goals for clinical research and be mentioned in labelling [31]. Examples include measuring the levels of fluoroestradiol F-18 by positron emission tomography (PET) to monitor the response of estrogen receptor (ER) positive lesions to endocrine therapy in patients with recurrent or metastatic breast cancer, and measuring the levels of phospho-AKT to monitor the inhibition of downstream phosphoinositide 3-kinase (PI3K) signalling in paired tumor samples to assess the target engagement of these drugs [32,33]. As opposed to direct assessments of how a patient feels, performs, or survives, surrogate endpoint biomarkers are endpoints utilized in clinical trials. Instead of evaluating the clinical benefit of main interest in and of itself, surrogate endpoints are intended to anticipate clinical benefit or harm in light of epidemiologic, pharmacological, pathophysiologic, or other scientific results. Response biomarkers may be considered candidate, reasonably plausible, or verified surrogate endpoints depending on the strength of the evidence supporting them. In addition to the production of medical products, response biomarkers can be used in clinical care settings. Response biomarkers' main function in clinical practice is to suggest a dose or method of administration.

G. Safety biomarkers:

In order to determine the possibility, presence, or severity of toxicity as a negative consequence, safety biomarkers are measurements taken before or after exposure to a medical product or an environmental contaminant. All safety biomarkers have the ability to detect or forecast adverse drug or exposure side effects. When a biomarker is found or changes, it may be possible to adjust the dose or stop the treatment before the toxicity gets worse. The safety biomarker could also indicate that a course of action is necessary in other situations. Additionally, safety biomarkers can be used to identify patients for whom a certain therapy shouldn't be started due to significant safety risks. Neutrophil count is used as a safety indicator to assess patients receiving cytotoxic chemotherapy in order to adjust the dose, determine when therapy must be stopped, or investigate the usage 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 urine, blood, stool, and, in less frequent cases, cerebrospinal fluid, saliva/buccal swabs, sputum, exhaled breath, and other body fluids (Figure 2). There is currently a focus on "liquid biopsies" because these biomarkers are an excellent substitute for collecting biopsies of specific organs for molecular analysis [1].

A. Blood:

Active secretion or cellular leakage from tumor cells or supportive tissues in the tumor surroundings might cause cancer biomarkers to enter the circulation. These circulating biomarkers, which can be used to assess tumor burden and spreading potential as well as give insight into molecular alterations in a tumor, include proteins and autoantibodies, nucleic acids such as cell-free DNA and RNA, circulating tumor cells, and microvesicles [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:

For clinical examinations and censuses, a fecal examination is the method that is most frequently employed. It is easy, non-intrusive, and ideal 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 extensively used 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]. There has been considerable interest in the possibility of DNA, RNA, proteins, microbes, and volatile chemical compounds in feces as biomarkers for CRC screening and early diagnosis. Because of their straightforward sampling and low risk, these biomarkers are anticipated to become a new focus for the next generation of CRC screening and early diagnostic tests [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 techniques have been used to locate cancer cells in biopsy samples of lymph nodes, solid tissue, bone marrow, peripheral blood, and other body fluids. Numerous studies have shown a substantial link between the PCR results and the existence of metastatic illness, as well as a high degree of specificity and sensitivity for tumor marker detection. PCR identifies tumor marker-expressing cells in patients with localized or metastatic cancer that would otherwise go undetected by standard techniques [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 discovered that the CR-LDR-qPCR assay can detect 30 methylated copies of each of the three BrCa-specific CpG markers when combined with an excess of unmethylated CpG markers (3000 copies each), which is a reasonable approximation of BrCa ctDNA overloaded 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 approach 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:

The study of biomarkers is increasingly using flow cytometry. Due to its multiparametric nature, it can provide incredibly accurate data on every single cell in a heterogeneous population. Both in preclinical and clinical settings, flow cytometry is utilized to produce biomarker data that can be used to inform decisions about clinical trial dose selection, cancer patient treatment options, and even a person's suitability for transplantation [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]. The FDA-approved gold-standard IHC biomarker for the detection of pancreatic cancer among diagnostic IHC biomarkers is carbohydrate antigen 19-9 (CA19-9) [67].

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

ELISA is still recognised as the gold standard for protein identification in physiological samples and has been widely used in routine clinical diagnostics. Because of its greater sensitivity, signal amplification, ease of use, automation potential, ability to be combined with miniature analytical systems, low price, and relative ease of mass manufacture, 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

Numerous therapeutic uses for cancer biomarkers exist, including risk assessment, screening, early detection, diagnosis, prognosis, response to therapy, cancer surveillance, and response monitoring [1]. These biomarkers can be used to evaluate individuals in a range of clinical situations, including risk assessment, primary cancer screening, identifying benign from malignant tissue, and cancer prognosis prediction. 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 attain precision medicine, which can aid in improving cancer treatment regimens as well as cancer detection and prevention strategies.

# CONCLUSION

Cancer cells endure several modifications, and these changes have been utilized as cancer biomarkers for decades, mostly in tumor tissue. New cancer biomarkers based on DNA, RNA, and proteins that can be found in readily available body fluids have been developed as a result of recent research on cancer biomarkers. Biomarkers play a vital 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.

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