Marking the Cancers: Importance of Biomarkers

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ABSTRACT

The term "biomarker" refers to a particular characteristic that can be used to evaluate responses to an exposure or intervention, pathogenic processes, or typical biological processes. Many diseases, including cancer, can be diagnosed with the help of biomarkers. The United States Food and Drug Administration (FDA) and the National Institutes of Health (NIH) collaborated to identify biomarkers and their categories, which are available publicly through an online document updated constantly 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. Cancer biomarkers can be studied in bodily fluids such as blood, stool, urine and less frequently, saliva/buccal swabs, exhaled breath, sputum, cerebrospinal fluid and other bodily fluids. Numerous methods can be employed to identify biomarkers. Fluorescence in situhybridization, Immunohistochemistry, Polymerase Chain Reaction, Enzyme-linked Immunosorbent Assay, Flow Cytometry, Microarrays, and Next Generation Sequencing are a few examples. Cancer biomarkers have a wide range of therapeutic applications, aiming to achieve precision medicine to maximize 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 are crucial in the diagnosis of many illnesses, 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 have several applications in oncology, such as estimating a person's risk of developing cancer, forecasting a treatment's chance of success for a particular patient, and tracking the course of a patient's illness to assess the efficacy of a treatment [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, miR-126, HE4, miR-92, miR-93, Mesothelin |
| Bladder | hOGG1, BTA, COX-2, miR-141-3p, miR-126, 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-141-3p, miR-10b-5p, ALK, KRAS |
| Liver | Α-Fetoprotein, miR-100-5p, HCCR-1, miR-122 |
| 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, through an online resource that is updated frequently, the “Biomarkers, Endpoints, and other Tools” (BEST). 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 show a person's chance of getting an illness or other medical condition even if they do not currently have one that is clinically evident. It is related to an elevated risk, or in certain conditions a lower possibility, 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, urinary concentration of tobacco-specific nitrosamines (TSNAs) for head and neck cancer and mutations in BRreast CAncer gene 1/2 (BRCA1/2) to determine a person's risk of developing ovarian and breast 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. These are employed to ascertain whether a patient is a candidate for a clinical trial investigating a specific disease or whether a patient has a specific medical condition for which therapy may be recommended. It is crucial to describe the anticipated function 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, progesterone receptor and human epidermal growth factor receptor-2 (ER/PR/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 one that is routinely assessed to determine the development 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 monitor the progression of the disease, which includes the appearance of new symptoms, the aggravation of preexisting anomalies, modifications to the disease's severity or a specific 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 evaluating drug metabolism, identifying therapeutic benefits or development of disease and determining 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 MGUS (monoclonal gammopathy of undetermined significance) are displaying symptoms of progression to various conditions [19,20,21].

D. Prognostic biomarkers:

Prognostic biomarkers identify people with the disease or medical condition of interest and indicate their likelihood of a clinical event, relapse of a disease, or development of disease. It indicates a greater or lesser likelihood of future clinical incidence, disease development, or recurrence in a certain group. Prognostic biomarkers are assessed at a baseline that has been predetermined and may include a background treatment. Prognostic biomarkers are frequently used in clinical settings where a patient has been identified with a disease or condition and it is desired to determine the chance of a subsequent clinical occurrence [22]. Prognostic markers are often used as eligibility criteria in clinical trials to diagnose patients who are more likely to have clinical events or to have their disease worsen. According to the United States Food and Drug Administration (2012), prognostic biomarkers are frequently used as factors of enrichment in pharmaceutical research and development. Some examples of prognostic biomarkers include BReast CAncer genes 1 and 2 (BRCA1/2) mutations for the assessment of breast cancer patients and the evaluation of the likelihood of developing a second breast cancer [23], deletions of chromosome 17p and mutations of TP53 for the evaluation of chronic lymphocytic leukemia patients and to determine the likelihood of death [24,25], increasing prostate-specific antigen (PSA) and Gleason to assess individuals with prostate cancer at the time of examination and to calculate the probability of developing cancer [26,27].

E. Predictive biomarkers:

Predictive biomarkers are those that can be used to identify individuals who, when exposed to a medicine or environmental pollutant, are more likely than identical individuals without the biomarker to experience a positive or negative reaction. They are used to identify individuals who are most likely to react negatively to a certain medication or environmental pollutant. The reaction 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 for decision-making related to patient care, such as identifying candidates for a particular treatment or choosing between multiple options. In the latter case, a description of the competing interventions under comparison should bolster evidence that a biomarker predicts the relative efficacy of an intervention. Predictive biomarkers for the effectiveness of interventions may include an individual's biological composition (referred to as "host characteristics"), the features of the sickness process, or other medical conditions [28]. A few examples include squamous differentiation in non-small cell lung cancer (NSCLC) to recognize patients who should avoid treatment with pemetrexed because it is likely to result in worse outcomes for survival or progression-free survival in comparison to conventional chemotherapies like cisplatin or docetaxel in combined with gemcitabine, BReast CAncer genes 1 and 2 (BRCA1/2) mutations for assessing women with platinum-sensitive ovarian cancer and to identify patients likely to react to Poly (ADP-ribose) polymerase (PARP) inhibitors [29,30].

F. Response biomarkers:

It is a biomarker used to show that an individual exposed to a medicine or environmental pollution has undergone a biological reaction, which could be beneficial or detrimental. They can be divided into two categories: pharmacodynamic biomarkers, which show the biological action of a drug or environmental factor without having to infer anything about the effectiveness or course of a disease or connecting this action to a known mechanism. These biomarkers could be employed as a proof-of-concept, to help choose the appropriate dose, or to quantify a reaction to drugs or environmental irritants, such as a gauge of possible risk. 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 matched tumor samples to assess the target engagement of these medications [32,33]. Surrogate endpoint biomarkers are clinical trial endpoints that are used instead of direct evaluations of a patient's feelings, abilities, or survival. Surrogate endpoints aim to predict clinical benefit or harm based on scientific findings related to pathophysiology, pharmacology, epidemiology, or other areas rather than assessing the clinical benefit of the main interest in isolation. Depending on the quality of the supporting data, response biomarkers can be classified as candidate, reasonably plausible, or validated surrogate endpoints. Response biomarkers have use in clinical care settings as well as in the manufacturing of pharmaceuticals. The primary purpose of response biomarkers 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 pre- or post-exposure to an environmental contaminant or medical product. 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 utilized to determine patients for whom a certain therapy shouldn't be started due to significant safety risks. Neutrophil count serves as a safety indicator to assess individuals receiving cytotoxic chemotherapy in order to modify the dosage, decide whether to discontinue treatment, 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 particular organs for molecular research [1].

A. Blood:

Active secretion or cellular leakage from tumor cells or supportive tissues surrounding the tumor 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, comprise polypeptides and autoantibodies, DNA and RNA, circulating tumor cells, and ectosomes [35].

B. Urine:

It has been demonstrated that urine is a reliable source of biomarkers in malignancies of the urinary tract, including kidney, bladder and prostate cancer [36]. Compared to blood biomarkers, which require the ability to relate peripheral markers to the intricate tumor microenvironment, urinary extracellular vesicles seem to be more centered as they are derived from proximate tumor tissues, share an 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 or FOBT, for example, is extensively used as a non-intrusive 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 nucleic acids, microorganisms, polypeptides and volatile chemical substances in feces as biomarkers for CRC examination. 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]. These biomarkers are expected to take on a new role in the development of CRC screening and early diagnostic tests due to their simple sampling and minimal risk.

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

Cancer biomarkers can be used for a variety of therapeutic purposes, such as risk assessment, screening, early detection, diagnosis, prognosis, response to therapy, cancer surveillance, and response monitoring [1]. These biomarkers can be used to assess people in a variety of clinical contexts, such as risk assessment, primary cancer screening, differentiating benign from malignant tissue, and predicting cancer prognosis. 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 tumor biomarkers for decades, mostly in cancer 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. New medications must endure rigorous evaluation and be tested in appropriately planned, randomized clinical studies in order to receive regulatory authorization. Unfortunately, despite the fact that biomarkers can have a significant impact on patient outcomes, such regulations do not exist. Therefore, it is important for clinical, translational, and laboratory researchers to be aware of the challenges in the development of biomarkers that are clinically relevant and transferable to the clinical setting. This will help to prevent the introduction of non-validated biomarkers that may be ineffective or even harmful to patient care.

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