Delivering on the promise of cancer biomarkers in the clinic

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Cancer is still the second leading cause of death in the United States and earlier diagnosis and effective therapies remain the holy grail of research paradigms. Cancer biomarkers have emerged as an invaluable tool in the achievement of this goal. Technological advancements and greater understanding of the molecular mechanisms of cancer have transformed biomarker research from an observational byproduct of cancer research into a biomedical research field in its own right. Despite the explosion of biomarker discovery over the last decade, few have been translated into clinical use. Here we discuss the current state of biomarker development and the challenges that have tempered their clinical potential.

Exploiting the unique cancer cell signature

Cancer continues to be a major cause of morbidity and mortality; in 2014, there will be an estimated 1.6 million new cases of cancer and more than half a million cancer-related deaths.[1] As such, there remains a pressing need for earlier diagnosis and improved treatment options.

Biomarkers are defined by the National Institutes of Health as "any characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention."[2] They have long been used as indicators of various disease states, and cancer is no exception. The first cancer biomarker, identified in the mid-1800s, was the immunoglobulin light chain, found in the urine of myeloma patients.[3] Since then, a variety of hormones, enzymes, and other proteins have been observed at altered concentrations in the biological fluids of cancer patients and have proven useful as biomarkers indicative of the presence of cancer.

Over the past several decades, significant technological advances and greater understanding of the molecular mechanisms underlying the development of cancer have led to the realization that the signature molecular alterations that drive the process of carcinogenesis are also an important source of potential cancer biomarkers (Figure 1). The result has been an explosion in cancer biomarker discovery and, although early discoveries were based primarily on empirical observations of single markers, there has been a shift toward large-scale analyses of multiple markers and the development of a multidisciplinary biomedical research field.

The promise of cancer biomarkers

As the field of cancer biomarkers has developed into its own entity, the potential clinical utility of biomarkers has likewise evolved giving rise to numerous types of cancer biomarkers (Table 1). A fairly comprehensive, though not exhaustive, list of biomarkers that are used in clinical practice and their approved uses is shown in Table 2. The vast majority of these biomarkers are protein-based, however, biomarkers encompass a wide range of different molecules, including deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNA), metabolites, and even whole cells.

Screening/diagnostic

Diagnostic markers can be present at any stage of cancer development and are designed to assist in providing a definitive diagnosis. Typically, cancer is diagnosed by examining the morphology of cells present in a biopsied tissue sample. Identifying variations in the levels of cancer biomarkers in biological fluids supplements the diagnosis by indirect characterization of the tumor. For example, in prostate cancer, increased levels of prostate specific antigen (PSA) in the blood, in combination with other clinical characteristics, are used to aid in diagnosis and staging.[4,5]

Recent advancements in high throughput genomic, proteomic, and even metabolomic technologies has driven the identification of DNA, RNA, protein, and metabolite biomarkers that are potentially informative in the diagnosis of cancer. Use of next-generation sequencing technologies can be particularly useful in establishing a diagnosis in metastatic tumors, for which there is frequent ambi-

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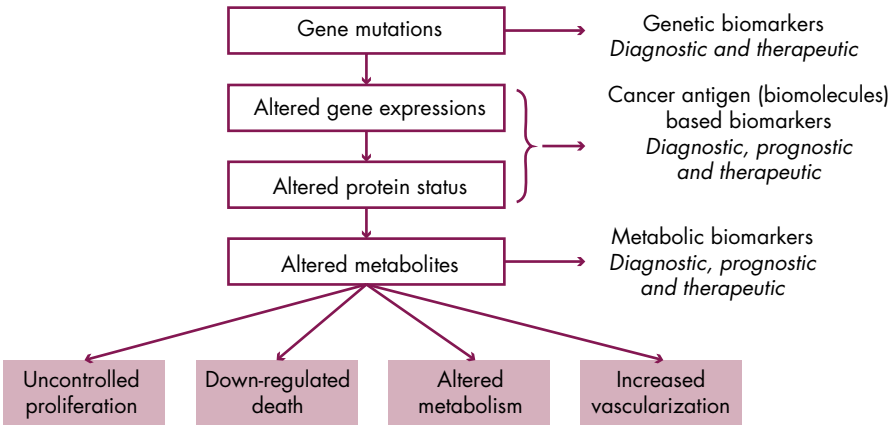

FIGURE 1 Opportunities for biomarker development at various stages of carcinogenesis

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guity. Each year in the US, between 90 and 130,000 newly diagnosed metastatic patients have an unclear diagnosis, many of which are so-called cancer of unknown primary.[6] By comparing the gene expression profile of a metastatic tumor sample with a database of known tumor types or subtypes, a more definitive diagnosis can be made. This is the basis of the CancerTYPE ID tool, which has shown almost 90% accuracy in distinguishing the tissue of origin in metastatic patients with unclear diagnosis.[7]

A significant goal is the identification of biomarkers, known as screening biomarkers, that are indicative of early-stage cancers, to assist in a more timely diagnosis. Thus far, most diagnostic biomarkers do not have adequate sensitivity or specificity for screening. One exception is the human papillomavirus, which is present in more than 90% of patients with uterine and cervical cancers and has formed the basis of a nationwide cervical cancer screening program.

Prognostic

Once a cancer diagnosis has been made, prognostic biomarkers are useful in determining the aggressiveness of the cancer type and predicting patient outcomes irrespective of treatment. A key example is the human epidermal growth factor receptor 2 (HER2) protein; high levels of HER2 expression are found on up to 20% of breast cancers and it is associated with increased tumor aggressiveness and reduced survival.[8-10]

There is an emerging realization that panels of biomarkers rather than single biomarkers will be required for biomarker assays to have sufficient sensitivity and specificity for diagnosis and prognosis. To this end, a number of multigene assays have been developed, some examples of which are shown in Table 3. The number of genes evaluated in these assays ranges from the single digits up to many hundreds. Perhaps best known are those used in breast cancer, such as the Oncotype DX test, which measures the expression of 21 breast cancer-associated genes in patients with ductal carcinoma in situ and invasive carcinoma to predict the likelihood of distant recurrence and the potential benefit of chemotherapy. Despite some controversy, the test has been incorporated into 3 major clinical guidelines in recent years.[11,12]

The most recently developed multigene assay is Prosigna, which determines the postsurgical risk of recurrence in patients with stage I/II node-negative or stage II node-positive and hormone receptor-positive patients. It incorporates the PAM50 expression profile of 50 genes, which classify the tumors into 4 intrinsic subtypes. Researchers evaluating the test found that it provided more prognostic information than other methods and was better able to distinguish between intermediate and high-risk patients.[13,14]

Predictive

Predictive biomarkers have been intensely investigated

TABLE 1 Types of biomarkers

Type	Description
Early detection/screening	Identifies cancer at an earlier stage than typical diagnostic methods
Diagnostic	Establishes a specific diagnosis
Disease monitoring	Assesses disease response during treatment, potentially allowing for adjustments
Risk assessment	Provide a quantitative means to determine predisposition to a certain type of cancer
Prognostic	Determine the aggressiveness of the particular cancer and predict outcome independent of specific treatment
Predictive	Predicts response to therapy and provides guidance in choice of therapy

TABLE 2 Biomarkers used in clinical practice

Biomarker	Clinical use
Human epidermal growth factor receptor 2 protein overexpression or gene amplification	Assessment for ado-trastuzumab emtansine and lapatinib therapy in breast cancer and for pertuzumab and trastuzumab therapy in breast and gastric cancer
EGFR exon 19 deletion or L858R (exon 21 substitution) mutation	Assessment for afatinib and erlotinib therapy in NSCLC
EGFR protein overexpression	Assessment for cetuximab and panitumumab therapy in CRC
Hormone receptor (estrogen receptor and progesterone receptor) expression	Assessment for anastrazole, everolimus, exemestane, fulvestrant, letrozole, and tamoxifen therapy in breast cancer
Philadelphia chromosome (t[9;22]) positive; BCR-ABL	Assessment for bosutinib, dasatinib, and nilotinib therapy in CML, ponatinib therapy in ALL, and imatinib therapy in CML and ALL
BCR-ABL T315I mutation	Assessment for ponatinib therapy in CML; drives resistance to other BCR-ABL targeting inhibitors
CD30 protein expression	Assessment for brentuximab vedotin therapy in Hodgkin lymphoma and anaplastic large cell lymphoma
KRAS codon 12 and 13 mutation	Identifies patients not eligible for treatment with cetuximab and panitumumab
Anaplastic lymphoma kinase gene rearrangement	Assessment for crizotinib therapy in NSCLC
BRAF V600E/K mutation	Assessment for dabrafenib, trametinib, and vemurafenib therapy in melanoma
CD25 antigen expression	Assessment for denileukin difitox therapy in cutaneous T-cell lymphoma
CD20 antigen expression	Assessment for ibritumomab tiuxetan therapy in B-cell NHL and follicular NHL, obinutuzumab and ofatumumab therapy in CLL, and rituximab therapy in CLL and NHL
c-KIT expression	Assessment for imatinib therapy in gastrointestinal stromal tumors
Platelet-derived growth factor receptor B gene rearrangement	Assessment for imatinib therapy in myelodysplastic/myeloproliferative disorders
Human papillomavirus	Screening for cervical cancer
HE-4	Monitoring of recurrence or progression of disease in ovarian cancer
CA-125	Prediction of malignancy as part of the OVA-1 and ROMA tests and monitoring disease progression and response to therapy in ovarian cancer by itself
Transthyretin	Prediction of malignancy in ovarian cancer as part of the OVA-1 test
Apolipoprotein A-1	Prediction of malignancy in ovarian cancer as part of the OVA-1 test
Beta microglobulin	Prediction of malignancy in ovarian cancer as part of the OVA-1 test
Transferrin	Prediction of malignancy in ovarian cancer as part of the OVA-1 test
Fibrin/fibrinogen degradation product	Monitoring disease progression in CRC
Alpha-fetoprotein L3 (AFP-L3)	Risk assessment for development of disease in hepatocellular carcinoma

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TABLE 2 *continued from page 383*

Biomarker	Clinical use
AFP	Management of testicular cancer
p63 protein	Aids in differential diagnosis in prostate cancer
CA19-9	Monitoring disease status in pancreatic cancer
CA15-3	Monitoring response to therapy in breast cancer
CA27.29	Monitoring response to therapy in breast cancer
Prostate-specific antigen	Prostate cancer diagnosis and monitoring; helps to discriminate between prostate cancer and benign disease
Nuclear mitotic apparatus protein	Diagnosis and monitoring of disease in bladder cancer
Circulating tumor cells	Prediction of cancer progression and survival in metastatic breast cancer, CRC, and castration-resistant prostate cancer
Bladder tumor antigen	Monitoring of bladder cancer
Thyroglobulin	Monitoring of thyroid cancer
Carcino-embryogenic antigen	Management and prognosis of cancer

ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer

Adapted from http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm and Füzéry22

because they have the potential to allow for truly personalized cancer therapy. More than 40 oncology drugs that have been approved by the US Food and Drug Administration include biomarker information in their labeling and FDA-approved companion diagnostics have been developed to test for these biomarkers (Table 4).

Once again a prominent example is the HER2 protein, which predicts response to the HER2-targeted therapies trastzumab, pertuzumab, ado-trastuzumab emtansine, and lapatinib in patients with breast and gastric cancer. Likewise, overexpression of the epidermal growth factor receptor (EGFR) is required for response to EGFR-targeted therapies, such as cetuximab, and panitumumab in patients with colorectal cancer (CRC). A number of genetic mutations and chromosomal rearrangements also serve as predictive biomarkers, such as mutations in the *BRAF* gene, which predict response to BRAF-targeted therapies, including vemurafenib.[15]

Predictive biomarkers are not only predictive of response, however, they can indicate that a patient will not respond to a particular therapy or that drug resistance has developed. Mutations in the *KRAS* gene generally indicate that a patient will not respond to EGFR-targeted therapy and as such these agents are only indicated in patients that screen negative for these mutations. Meanwhile, a specific mutation in the *BCR-ABL* gene (T315I) in patients with chronic myelogenous leukemia is indicative of resistance to BCR-ABL targeting inhibitors. As a result of the identification of this biomarker in resistant patients, second-generation agents such as ponatinib have been developed that are effective even in the presence of this mutation.[16]

Novel biomarker strategies

In recent years, a number of novel types of cancer biomarker have been identified. Two in particular that are receiving significant attention are circulating tumor cells (CTCs) and circulating cell-free nucleic acids (cfNA). Although the former are approved as prognostic biomarkers in metastatic breast cancer, CRC and castration-resistant prostate cancer, cfNAs are still in early development.

CTCs are isolated tumor cells that have broken away from the site of disease in metastatic and/or primary cancers. Research has shown that CTCs could serve as valuable noninvasive prognostic biomarkers, dubbed a "liquid biopsy," offering insight into the formation of metastases at an earlier stage than do the current high-resolution imaging technologies. High basal levels of CTCs in patients with metastatic breast cancer, CRC, and prostate cancer have been found to correlate with poor prognosis.[17]

Since CTCs are present in the range of only a few cells per millimeter of blood, even in patients with advanced metastatic cancer, the challenge of using CTCs is to identify them above a background of normal blood cells, so most methods for the identification of CTCs involve an initial enrichment step.[18] Numerous methods have been developed that typically focus on isolating the CTCs on the basis of physical (eg, size) or biological (eg, presence of tumor-associated antigens) properties. Currently, the only FDA-approved method for CTC enrichment and identification is CellSearch, which uses magnetic particles coated with antibodies against the epithelial-specific antigen, epithelial cell adhesion molecule (EpCAM). However, many other methods are in clinical development.[19]

TABLE 3 Multigene expression panels

Test	Manufacturer	Description	Clinical application
Ova1	Aspira Labs	Qualitative serum test that combines the CA-125 and HE4 tests in ovarian cancer tissue samples combined with menopausal status	Determines the likelihood of finding a malignancy on surgery in women who present with ovarian adnexal mass
Risk of Ovarian Malignancy Algorithm (ROMA)	Fujirebio Diagnostics	Qualitative serum test that examines the expression of five biomarkers in ovarian cancer tissue samples; beta-microglobulin, CA-125, apolipoprotein A1, prealbumin, and transferrin	Evaluates an ovarian mass for malignancy prior to planned surgery
Oncotype DX	Genomic Health	Biopsy-based RT-PCR assay that examines the expression of 17 genes (prostate cancer), 12 genes (colon cancer) or 21 genes (breast cancer) in tumor tissue samples	Predicts cancer aggressiveness in prostate cancer, predicts risk of recurrence in patients with stage II/III colon cancer, guides treatment decisions in patients with ductal carcinoma in situ or invasive carcinoma
Prolaris	Myriad Genetics	Examines the expression of 31 cell cycle progression genes in prostate cancer tissue samples	Identifies low-risk patients with prostate cancer and estimates risk of recurrence and guides therapeutic adjustment in patients with high-risk features after surgery
MammaPrint	Agendia	Analyzes the expression of 70 genes in early-stage breast cancer tissue samples	Identifies patients with high risk of distant recurrence and guides therapeutic decision-making
TargetPrint	Agendia	Microarray-based gene expression test used in breast cancer tissue samples	Offered in conjunction with MammaPrint to provide qualitative assessment of patient's estrogen receptor, progesterone receptor and HER2 expression levels
BluePrint	Agendia	Multigene profile used in breast cancer tissue samples	Separates breast tumors into molecular subtypes; basal-type, luminal-type, and HER2-type
Prosigna™	NanoString Technologies	Uses the PAM50 test to examine the expression of 50 genes in breast cancer tissue samples	Determines postsurgical risk of recurrence in postmenopausal women with stage I/II node-negative or stage II node-positive and hormone receptor-positive breast cancer
CancerPRS	Signal Genetics	Examines the expression of 163 genes (ColonPRS) or 200 genes (BreastPRS) in tumor tissue samples	Predicts recurrence and overall survival in patients with stage II/III colon cancer (colon PRS) and identifies risk of recurrence for up to 10 years in breast cancer patients (BreastPRS)
Coloprint	Agendia	Measures the expression of 18 genes in colon cancer tissue samples	Identifies risk of distant, local or regional relapse in patients with early-stage colorectal cancer
Genefx colon	Precision Therapeutics	Microarray-based assay to examine the expression of 634 DNA transcripts in colon cancer tissue samples	Assesses risk of recurrence within 5 years in patients with stage II colon cancer
Oncodefender CRC	Everist Genomics	Examines the expression of 5 genes in colorectal cancer tissue samples	Assesses risk of recurrence in stage I and II colorectal cancers and guides therapeutic decision-making

cfNAs, including DNA, RNA, and microRNA, are released from tumors into the blood stream when tumor cells undergo necrosis or apoptosis and may even be secreted by cancer cells. Altered levels of cfNAs are associated with increasing tumor burden and malignant progression. As with CTCs, cfNAs could also provide a liquid biopsy, and they are being evaluated as biomarkers of cancer progression and metastasis, as well as in cancer

TABLE 4 Companion diagnostics approved by the US Food and Drug Administration

Device	Manufacturer	Description	Clinical application
Therascreen KRAS RGQ PCR Kit	Qiagen	Real-time quantitative PCR assay that detects seven somatic mutations in the *KRAS* gene using DNA extracted from FFPE CRC tissue	Identifies patients who are eligible for treatment with cetuximab and panitumumab on the basis of a 'no *KRAS* mutation' result
DAKO EGFR PharmDx Kit	Dako	IHC assay that identifies EGFR expression in CRC patients	Identifies patients who are eligible for treatment with cetuximab and panitumumab on the basis of EGFR expression positivity
Therascreen EGFR RGQ PCR Kit	Qiagen	Real-time PCR assay for the qualitative detection of exon 19 deletions and exon 21 (L858R) substitution mutations in the *EGFR* gene in DNA derived from FFPE NSCLC tissue	Identifies patients in whom afatinib is indicated
Cobas EGFR Mutation Kit	Roche Molecular Systems	Real-time PCR test for qualitative detection of exon 19 deletions and exon 21 (L858R) substitution mutations of the *EGFR* gene in DNA derived from FFPE NSCLC tumor tissue	Aids in the selection of patients with metastatic NSCLC who are eligible for treatment with erlotinib
INFORM HER2/NEU	Ventana Medical Systems	FISH DNA probe assay that determines the qualitative presence of *HER2/neu* gene amplification in FFPE breast tissue	Aids stratification of breast cancer patients according to risk for recurrence or disease-related death; used as an adjunct to existing clinical and pathologic information
PATHYVISION HER2 DNA Probe Kit	Abbott Molecular	FISH assay designed to detect amplification of the *HER2/neu* gene in FFPE breast cancer tissue	Used as an adjunct to existing clinical and pathologic information currently used as prognostic factors in stage II, node-positive breast cancer; aids in prediction of disease-free and overall survival in patients with stage II, node-positive breast cancer treated with adjuvant cyclophosphamide, doxorubicin, and 5-fluorouracil and in assessment of patients being considered for trastuzumab treatment
PATHWAY Anti-HER2/NEU (4B5) Rabbit Monoclonal Antibody	Ventana Medical Systems	Mouse monoclonal antibody for semi-quantitative detection of HER2/neu in FFPE breast tissue	Aids in the assessment of patients being considered for trastuzumab treatment
INSITE HER2/NEU Kit	Biogenics Laboratories	Mouse monoclonal antibody (Clone C1B11) for the semi-quantitative localization of HER2/neu overexpression by light microscopy in FFPE breast tissue	Aids in the assessment of patients being considered for trastuzumab treatment
SPOT-LIGHT HER2 CISH Kit	Life Technologies	CISH assay intended to quantitatively determine *HER2* gene amplification in FFPE breast cancer tissue	Aids in the assessment of patients being considered for trastuzumab treatment; adjunct to the clinicopathological information currently used for the management of breast cancer
Bond Oracle HER2 IHC System	Leica Biosystems	Semi-quantitative IHC assay that determines HER2 protein status in FFPE breast cancer tissue	Aids in the assessment of breast cancer patients being considered for trastuzumab treatment
HER2 CISH PharmDx Kit	Dako	Dual color CISH assay that quantitatively determines *HER2* gene status in FFPE breast cancer tissue	Aids in the assessment of patients being considered for trastuzumab treatment; adjunct to clinicopathologic information currently used for estimating prognosis in stage II, node-positive breast cancer patients
INFORM HER2 DUAL ISH DNA Probe Cocktail	Ventana Medical Systems	Dual ISH DNA probe cocktail that determines *HER2* gene status in FFPE breast cancer tissue	Aids in the assessment of patients for whom trastuzumab treatment is being considered

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TABLE 4 *continued*

Device	Manufacturer	Description	Clinical application
HERCEPTEST	Dako	Semiquantitative IHC assay that identifies HER2 protein overexpression in FFPE breast and gastric cancer tissue	Aids in the assessment of breast and gastric cancer patients being considered for trastuzumab treatment and for breast cancer patients being considered for pertuzumab or T-DM1 treatment
HER2 FISH PharmDx Kit	Dako	Direct FISH assay designed to quantitatively determine *HER2* gene amplification in FFPE breast and gastric cancer tissue	Aids in assessment of breast and gastric cancer patients being considered for trastuzumab treatment and breast cancer patients being considered for pertuzumab or T-DM1 treatment; adjunct to clinicopathologic information currently used to estimate prognosis in stage II, node-positive breast cancer
THxID™ BRAF Kit	bioMérieux	Real-time PCR test that qualitatively detects the *BRAF V600E/K* mutations in DNA samples extracted from FFPE melanoma tissue	Aids in selection of melanoma patients for treatment with dabrafenib and trametinib
Cobas 4800 BRAF V600 Mutation Test	Roche Molecular Systems	Real-time PCR assay designed to qualitatively detect the *BRAF V600* mutation in DNA extracted from FFPE melanoma tissue	Aids in the selection of melanoma patients eligible for treatment with vemurafenib
DAKO c-KIT PharmDx Kit	Dako	IHC assay that identifies c-KIT protein expression in patients with gastrointestinal stromal tumors	Allows differential diagnosis of GIST and identification of patients eligible for treatment with imatinib
VYSIS ALK Break Apart FISH Probe Kit	Abbott Molecular	FISH assay that qualitatively detects *ALK* gene rearrangements in FFPE NSCLC tissue specimens	Aids in identification of patients eligible for treatment with crizotinib

CISH, chromogenic in situ hybridization; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescent in situ hybridization; IHC, immunohistochemical; NSCLC, non-small-cell lung cancer; T-DM1, ado-trastuzumab emtansine

Adapted from: http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm

screening and monitoring therapeutic responses.[20,21]

Challenges from bench to bedside

Despite the recent boom in biomarker discovery, very few actually make it into clinical practice. There are several key phases of biomarker development and numerous challenges present at each stage that can prevent progression to the next. The most important factors in the clinical acceptance of a biomarker are the magnitude of its clinical value and the quality of clinical trial data. As such, these are areas where biomarker development typically runs into difficulty as researchers face hurdles in identifying the true clinical utility or lack well-controlled trial data (Figure 2).

The effective clinical validation of a biomarker is extremely complex, time consuming, and expensive. Because biomarkers were initially often identified as a byproduct of research, one of the most significant confounding issues in their effective translation into the clinic was a limited understanding of optimum analytical, diagnostic and regulatory requirements for biomarker validation. With the evolution of the biomarker field into a bona fide area of research this has begun to change. Researchers in the field are developing a framework for effective biomarker development that includes the implementation of clinical guide-

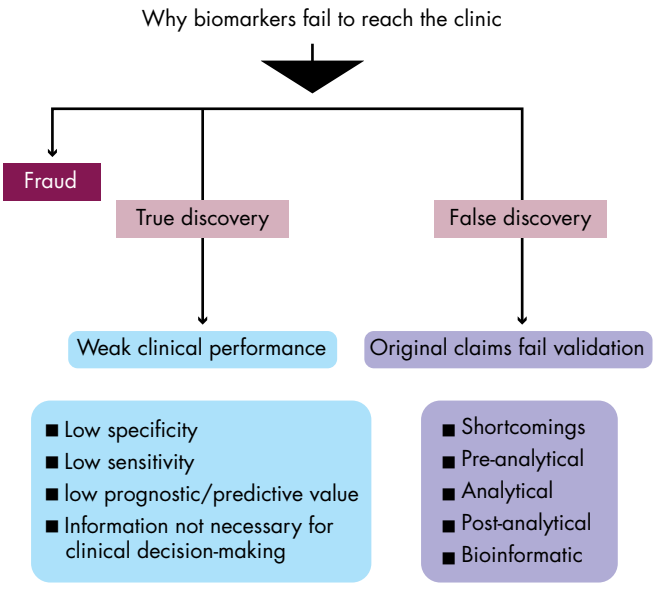

FIGURE 2 Summary of the reasons for biomarker failure to reach the clinic

Adapted with permission from Diamandis EP. BMC Medicine. 2012;10:87.

lines (eg, REMARK [Reporting Recommendations for Tumor Marker Prognostic Studies] guidelines).[15,22,23]

The road from bench to bedside for cancer biomarkers is long and arduous, but new and exciting discoveries continue to be made. As researchers begin to understand the challenges faced and develop strategies to overcome these barriers, cancer biomarkers may begin to meet their full potential in personalized cancer therapy.

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