

Making Meaningful Clinical Use of Biomarkers

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ABSTRACT: This review discusses the current state of biomarker discovery for the purposes of diagnostics and therapeutic monitoring. We underscore relevant challenges that have defined the gap between biomarker discovery and meaningful clinical use. We highlight recent advancements in and propose a way to think about future biomarker development.

KEYWORDS: Biomarkers, liquid biopsy, diagnostics

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Introduction

Personalized medicine is “big data” and the data explosion continues. As we unravel the genome to look for individualized clues, we are faced not only with just more than 3 billion base pairs but also with epigenomic changes, noncoding regions, regulatory sequences, etc, that add layers of complexity. New technologies continue to be advanced to identify molecular markers of disease within this information. Although tools have made identifying variants a fashionable pursuit, identifying *actionable* variants that serve as biomarkers has the potential to revolutionize the way health care is delivered in the future.

This writing is somewhat divergent from the descriptive approach of outlining exciting new technologies and listing examples of various micro RNAs that have been identified as potential biomarkers. The diligent work of many has created a wealth of data that no single expert can keep up with.^{1,2} We hope rather to discuss here the current state of biomarker use in various forms and the shortcomings of our current translational models and their potential paths forward.

A biomarker refers to a quantifiable biological parameter that is measured and evaluated as an indicator of normal biological, pathogenic, or pharmacologic responses to a therapeutic intervention, as defined by the National Institutes of Health.³ Accordingly, glomerular filtration rate, repeat blood pressure readings, hemoglobin A_{1C}, and gene expression profiling are all examples of “biomarkers.” When used in translational research discussions, the term itself often alludes to a marker used to accelerate or aid in diagnosis or monitoring and provide insight into “personalized” medicine.

Furthermore, a “liquid biopsy,” as it is starting to be called, could add significant clinical value. This noninvasive, or minimally invasive, biomarker testing could allow for rapid, economical, and repeat evaluation.⁴ This repeat sampling feature would thus allow for the patient with high potential for a particular disease to self-sample urine and saliva or for clinical

sampling of serum/plasma or whole blood. To date, most of the liquid biopsy research has focused on the rare circulating tumor cell or CTC; it is proposed that nucleic acids and proteins, either free or found in extracellular vesicles such as exosomes,⁵ may prove useful.⁴

A search of the relevant literature will reveal an abundance of publications related to biomarkers.⁶ Burke reported in 2016 that he had identified more than 768 000 papers indexed in PubMed.gov directly related to biomarkers.¹ Despite advances in laboratory technology and an enormous expansion in relevant literature, we seem quite far from widespread clinical use of biomarkers in discovery, treatment, or monitoring. In fact, in cancer, for example, today there exists only a few dozen clinically relevant biomarkers in use.^{7–14}

The latest European Society of Medical Oncology (ESMO) clinical practice guidelines for lung, breast, colon, and prostate cancers give a weak recommendation for the use of no less than 20 molecular markers.^{7–11} Even more, the ESMO clinical practice guidelines for pancreatic cancer, for example, state that there is no relevant biomarker used in the medical decision making and none should be used in clinical practice.¹⁵ Granted, they do go on to list several recent studies highlighting pancreatic cancer biomarkers of interest including STK11, ERBB2, MET, CDK6, PIK3CA, BRCA2, PALB2, MLH1, MSH2 and SMAD4, KRAS, and CDKN2A, among others. This lack of strong support from clinical practice guidelines certainly does not nullify their value. These guidelines are often thought of as the benchmark minimums for delivering care, and biomarkers are not yet part of a larger diagnostic and therapeutic framework but rather exist largely in the realm of research.

It is definitely not our intent to present a pessimistic view of the current use of biomarkers, as they have for some time played invaluable roles in the diagnosis, early detection, and effective treatment of various maladies, particularly in cancer as



we will later mention (Table 1). The management of leukemia and lymphoma, for example, has been positively affected using anti-CD20.^{16,17} Another example is the ALK gene and its significance in lung cancer. Originally discovered in 1994 for its association with anaplastic large cell lymphoma, in 2007, it was found to be a molecular driver of non-small-cell lung cancer. In 2011, crizotinib (Xalkori; Pfizer, Inc., New York, NY) was approved to treat late stage lung cancer expressing the abnormal ALK gene. We do, however, want to highlight some of the challenges that the field has faced and look forward to what may come.

Diagnostic Biomarkers

The pace at which biomarker discovery is translated into clinical use is often slow and arduous. A historical illustration of this lies within the story of the Bence Jones protein described by Schiess et al.¹⁸ In 1847, Bence Jones discovered a peculiar protein in the urine of a patient with multiple myeloma.¹⁹ More than 100 years later, it was identified as a free antibody light chain produced by the tumor.²⁰ In 1988, more than 140 years later, a routine diagnostic test for the protein as a biomarker for multiple myeloma was approved by the United States Food and Drug Administration (FDA).²¹

Often, challenges other than time underscore the gap between discovery and meaningful use. To truly be valuable, a biomarker must contribute clinically relevant information beyond what is available or provide the same information at a lower cost, either financially or in measurable patient risk. As a tool for improved diagnostics, molecular signatures can be used to increase diagnostic accuracy in the general population, at-risk populations, or those with suspicious clinical findings that require confirmation. Using pancreatic cancer as a model, cancer antigen 19-9 (CA19-9) has an approximately 80% sensitivity and 90% specificity.²² In addition, it is useful for prognostication and detecting recurrence. It is, however, elevated in additional pathologies, such as biliary obstruction which often coexists with pancreatic cancer. In addition, it lacks the predictive value required to be a stand-alone diagnostic biomarker, particularly in healthy individuals.^{22,23} In 70 940 asymptomatic patients, Kim et al²³ found a 0.9% positive predictive value for detecting pancreatic cancer. Numerous newer disease signatures have been identified as mentioned earlier, but none has been clinically validated for routine use. Several additional reviews have addressed the misconceptions and shortcomings that contribute to the void between discovery and meaningful clinical use to replace current reference standards.^{1,24,25}

Additional recognizable markers include carcinoembryonic antigen used in colorectal cancer surveillance, prostate-specific antigen which is elevated in prostate cancer, CD20 which is useful in recognizing and treatment of relapsed and/or refractory follicular lymphoma,¹⁷ and procalcitonin that is used to monitor response to antibiotics in patients with sepsis.^{26,27} Even

these well-established tools have been criticized. Some of them were developed prior to the level of sophistication we have today in molecular biology as well as clinical trials. Underpowered studies, lack of high sensitivity and specificity, and a propensity for overuse leave room for increased precision.^{28,29}

Challenges such as poor study design, complicated statistical analyses, lack of prospective studies, poor reproducibility, and inability to translate “benchwork” to the clinic may limit success of prospective markers. Genetic tests for clinical and public health use, are expected to pass evaluation of their analytical validity, clinical utility, and clinical validity, as set for by the Evaluation of Genomic Applications in Practice and Prevention Initiative³⁰ (Table 2). We would suggest that applying these same measures to biomarker design, discovery, and application may improve success with a vision of clinical use.

Discovery experiments, often referred to as “fishing expeditions,” illustrate some of the challenges mentioned above. This often begins with available archived samples followed by investigations such as proteome sampling, which reveals mostly highly expressed proteins such as albumin and immunoglobulins. This is at odds with identifying a very sensitive and specific biomarker that is diluted by an abundance of peptides.^{18,31} In addition to technical difficulties, simple variation in sex, or time of day, as well as concomitant medical conditions or treatments, may alter the proteome within individuals.^{4,32} Reproducibility has been challenging for many as the composition of circulating proteins in the blood represents what is happening to the whole organism at any given time.¹⁸ It is therefore important to understand that the information obtained will be only snapshot of the organism at a specific time. The added natural complexity of cancer itself limits the insight a single molecular signature provides. Collectively, these elements contribute to the complexity of deriving meaningful data from investigations.

The development of a clinically meaningful diagnostic biomarker best begins with a predefined roadmap. At the beginning of this is a central question: “Is there an unmet clinical need?”. As potential candidates develop, a reproducible assay must be available and used to define the markers distribution in the target population. This should then be tested against the “gold standard” for diagnosis. If added diagnostic accuracy exists, then biomarker performance should be validated.³³ In “Predicting Clinical Outcomes Using Molecular Biomarkers,” Burke set forth a thorough review of the framework for proposing, validating, and using biomarkers.¹ He outlines several methodologic flaws and common misconceptions. His writing underscores the importance of a strong a priori understanding of limitations and end points.

Central to discovery is study design, part of which includes sample acquisition. Oftentimes, subject selection is driven more by specimen availability rather than a stringent study protocol. This retrospective examination is not only subject to immense bias but may also lack thorough patient data for

Table 1. Common predictive and diagnostic biomarkers used in treatment decisions in cancer.^{a,b}

BIOMARKER	CANCER TYPE	SOURCE
CD20	B-cell lymphoma, leukemia	Blood, marrow
21-gene RT-PCR	Breast	Tissue
ER/PR	Breast	Tissue
CA15-3	Breast	Blood
CA27-29	Breast	Blood
HER-2/NEU	Breast, esophagogastric	Tissue
BRCA1/2	Breast, ovarian, prostate, pancreatic	Saliva, serum
CEA	Colon, medullary thyroid, stomach	Blood
RAS	Colon, NSCLC	Tumor
MLH1, MSH2, MSH6, PMS2	Colon, NSCLC, esophagogastric, HNSCC	Tumor
BRAF	Colon, NSCLC, melanoma, papillary thyroid, leukemia, glioma	Tumor
PD-L1	Colon, NSCLC, soft tissue sarcoma	Tumor
cKIT/CD-117	GIST, soft tissue sarcoma	Tumor
PDGFRA	GIST, soft tissue sarcoma	Tumor
BCR-ABL1	Leukemia	Blood, marrow
FLT3	Leukemia	Blood, marrow
P53	Leukemia	Blood, marrow
NPM1	Leukemia	Blood, marrow
CEBPA	Leukemia	Blood, marrow
AFP	Liver	Blood
ALK	NSCLC	Tumor
EGFR	NSCLC	Tumor
ROS1	NSCLC	Tumor
CA-125	Ovarian	Blood
CA19-9	Pancreatic	Blood
PSA	Prostate	Blood

Abbreviations: AFP, alpha-feto protein; ALK, anaplastic lymphoma receptor tyrosine kinase; BRAF, RAF-protooncogene; CEA, carcinoembryonic antigen; CEBPA, CCAAT/enhancer binding protein α ; RT-PCR, reverse transcription-polymerase chain reaction; EGFR, epidermal growth factor receptor; ER/PR, estrogen receptor/progesterone receptor; FLT3, Fms-related tyrosine kinase 3; GIST, gastrointestinal stromal tumor; HNSCC, head and neck squamous cell carcinoma; NPM1, nucleophosmin 1; NSCLC, non-small-cell lung cancer; PDGFRA, platelet-derived growth factor receptor α ; PSA, prostate-specific antigen; RAS, rat sarcoma; ROS, protooncogene.

^aDerived from National Comprehensive Cancer Network (NCCN). The NCCN Biomarkers Compendium (NCCN Compendium). <http://www.nccn.org/professionals/biomarkers/content/>. Accessed May 12, 2017.

^bDerived from Table of Pharmacogenomic Biomarkers in Drug Labeling. Food and Drug Administration. <https://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm>. Accessed May 12, 2017.

support and be unable to satisfy power calculations based on subject inclusion criteria. This raises concerns of clinical validity as well as future clinical utility. Given the limitations of prospective studies, it makes practical sense to begin with archived specimens. Although this has not been traditionally accepted as high-quality evidence, some experts have proposed scenarios in which using archived specimens to validate biomarkers may be

of considerable value.³⁴ Emphasis has been placed on “prospective-retrospective” studies, assaying samples from previously conducted prospective trials. For example, genomic analysis of samples collected during a phase 2 trials can be used to identify a companion diagnostic marker. This can later be applied to a phase 3 trial to decide which patients would benefit most from a chosen treatment. With more robust results, due to improved

Table 2. Genomic and suggested biomarker evaluation parameters.

Analytical validity	Reproducibility; is the test accurate?
Clinical validity	Are the results medically meaningful; can a biomarker distinguish one group from another in a meaningful manner?
Clinical utility	Does a test improve health care; will the results of a test change outcomes?
Other (cost-effectiveness, psychological implications, ethical implications)	Is there value added or cost saved by knowing the results? Do we have a treatment or risk reduction strategy to implement based on results?

Adapted from Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Initiative³⁰ with modifications.

patient selection, drug manufacturers, patients, and physicians all stand to benefit from streamlined drug approval, tailored therapies with less side effects, more cost-effective care, and better outcomes.

This elevates the role of the pharmaceutical industry at each step. Large, well-designed prospective trials are costly, and the deep pockets of big pharma can support them. The disease process of systemic lupus helps illustrate this. Industry took notice of a medical need for improved therapies and made investments in discovery. There are now a number of new medications in clinical trials as well as newly FDA-approved ones. This created a scenario in which novel biomarkers may help define subsets of patients likely to respond to certain treatments.³⁵ Correctly identifying such a group not only benefits those taking the medication but also future trials that may recruit more homogenous subjects creating more robust results.

Enlisting industry support carries with it involvement of various regulatory bodies. Guidance for much of this process has been set forth by the FDA, who has published documents for industry guidance and letters of support that encourage further evaluation of biomarkers being pursued.^{36,37} In drug development and clinical trials, biomarkers may be used to help identify populations for a study, monitor therapeutic response, and identify side effects. The FDA's Center for Drug Evaluation and Research defines the biomarker qualification process for this use.³⁶ In addition, they impose rules on the use of "in vitro companion diagnostic devices," such as next generation sequencing and the clinical validity of other technologies used for biomarker detection. When used in research, they are generally considered "investigational" and if "significant risk" is involved, they must receive an "investigational device exemption" from the FDA.³⁸ Layers of regulatory burden can be difficult for both basic scientists and clinicians to navigate.

Reaching the market attracts additional interest. In November of 2013, the FDA ordered 23andMe, Inc., a direct-to-consumer personal genetics company, to cease and desist from providing consumers with personal "health risks" and "drug response" information. This stood until the tests' accuracy could be clinically validated.³⁹ They originally provided likelihood assessment on 254 diseases and conditions from saliva samples sent via the mail, but in compliance with 2015 FDA guidance, they now provide approximately 4 "genetic health risk" and 40 "carrier status" reports, on their Web site.⁴⁰ They

now only report if the consumer has a single copy of a genetic variant related to disease or a "carrier status." In addition, they require that 23andMe provide information to consumers about how to obtain access to a board-certified clinical molecular geneticist or equivalent if the product is sold over the counter.

This caught national attention as the questions raised go well beyond those of clinical validity. They stimulated discussion about the ethical implications of providing patients with information they are not equipped to completely understand. These tests are not similar to over-the-counter pregnancy tests which provide a "yes" or "no" answer. Instead, they indicate what it means to an individual patient to be a "carrier," or even further, to carry a variance of uncertain significance? The implications of these results may be far reaching. What are the risks of false positives, false negatives, and even true results? If clinicians are not certain what to do with much of this information, how best can consumers be educated?

Predicting Response and Therapeutic Monitoring

Although these complexities have slowed progress in the realm of diagnostics, biomarkers serve multiple purposes. Their use in therapeutic monitoring is taking several forms including predicting response, monitoring for side effects, and as surrogate endpoints.⁴¹ The Biomarkers, EndpointS, and other Tools statement from the National Institutes of Health explains that in addition to predicting response, biomarker monitoring during the course of an intervention serves several roles. Several of these end points include monitoring for therapeutic range of a prescribed treatment, detecting therapeutic response, and guiding additional therapies, or changes in treatment.

Treatments may be assigned to large numbers of patients, despite uncertainty in the degree of their response to therapy. An example of this is the systemic chemotherapy offered to locally patients with advanced pancreatic cancer with response rates ranging from single digit to approximately 30%.⁴² The routine use of adjuvant chemotherapy for rectal cancer following neoadjuvant chemoradiation is being questioned.⁴³ This overtreatment in some cases, or "trial and error" approach in others, is lacking in precision, carries with it side effects, the possible progression of disease, and is seen by many as unreasonably expensive. Ideally, therapeutic biomarkers should be used to guide such critical treatment decisions.

Progress certainly has been made in several malignancies. Lymphoma, for example, has been treated based on the presence or absence of the CD20 antigen on the surface of B-cell non-Hodgkin lymphoma (NHL). The CD20 antigen monoclonal antibody rituximab⁴⁴ (IDEC Pharmaceuticals, La Jolla, CA, USA) was shown to be an effective single-agent treatment for follicular NHL. In February of 2002, the FDA approved the use of 90Y-ibritumomab tiuxetan (IDEC Pharmaceuticals) as the first commercially available radiolabeled antibody for cancer treatment.⁴⁵

In another example, breast cancer has been treated based on the presence or absence of several biomarkers including estrogen receptor and progesterone receptor (ER/PR) status, and human epidermal growth factor receptor status (HER2). Numerous additional molecular markers have been linked to breast cancer and have appeared in the clinical literature including gene mutations, messenger RNAs, CYP450 polymorphisms, CTCs, and gene panel testing.⁴⁶ In 2007, a commercially available genetic testing platform, OncotypeDX[®] (Genomic Health Inc., Redwood City, CA, USA) was the first FDA-approved multivariate molecular test. It uses the expression pattern of 21 genes in paraffin-embedded tumor tissue to predict the likelihood of distant recurrence in patients with node-negative, tamoxifen-treated breast cancer.⁴⁷ The recognition of the heterogeneity of breast cancer creates a valuable paradigm to think about diagnostic and therapeutic biomarker development and use. Similar genetic panel testing is a tool that will likely inform additional diagnosis and treatment decisions as we move forward.

Futuristic Outlook

The federal government, professional societies, and the public media believe that we will experience a future informed by personalized medicine. The expectations are high and maybe even somewhat unrealistic if we consider article headlines from more than 10 years ago when the human genome was sequenced. The FDA maintains a publicly available database of biomarkers listed on drug labels. In a survey reported in *Clinical Pharmacology and Therapeutics* in 2012, Stanek et al⁴⁸ reported that approximately 10% of physicians felt adequately informed about pharmacogenomics testing. This was despite 97.6% agreeing that genetic variations may influence drug response. The gap between biomarker discoveries and this type of clinical use we hope for can only be narrowed with a translational approach. We will need to reexamine incentives and move toward collaborative innovation.

We may benefit from looking outside of medicine for assistance. The rate of biomarker discovery has outpaced our ability to validate them and those with knowledge and experience in *big data* need to be involved.⁴⁹ Internet commerce and social media platforms are examples of other industries that have positioned themselves as data companies. Although Tesla, the revolutionary electric car with self-driving capabilities, has crashed on several instances, complicated machine learning

algorithms collect observations from data over time and continue to improve the cars performance.⁵⁰ Medicine, however, has long been criticized for lagging behind other industries in both innovation and adoption. Co-opting machine learning will become a necessity to aid in discovery and deliver higher quality health care. Recognizing this, will help us leverage collaboration to overcome the “bumps in the road” as we explore the complexity of prediction in medicine.

Fast forward a few decades from the story of Bence Jones to the initiative “Cancer Moonshot 2020.” According to its Web site, in October 2015, Dr Patrick Soon-Shiong, a billionaire investor and surgeon met with Vice President Joe Biden and presented a position paper titled, “The Precision Against Cancer PAC; The Moonshot Program to Develop a Cancer Vaccine for “I am N = 1.”⁵¹ This collaborative project aims to enroll 20,000 patients with 20 different tumor types in phase 2 cancer vaccine trials. A few weeks later, Vice President Joe Biden gave a speech calling for a “moonshot” to cure cancer. The Cancer Moonshot, managed by a consortium of companies called The National Immunotherapy Coalition, is a public-private partnership consisting of the government (NCI, FDA, White House, and Congress), pharmaceutical and biotech industry, health care providers, and insurance companies that have aligned to accelerate 10 years of advancement into 5 years.⁵² Several pillars of their work include big data analytics, next generation sequencing, and predicting response to treatments.

Along with unified support comes large dollar funding. As part of the 21st Century Cures Act signed into law in December 2016, \$1.8 billion was allocated for the Cancer Moonshot and \$1.4 billion for the Precision Medicine Initiative aimed at collecting the genetic information of 1 million Americans.⁵³ If appropriated each year, this funding demonstrates commitment to the importance of finding meaningful biomarkers.

Recently, Guardant Health, with a patented digital sequencing platform, joined forces with AstraZenica, Merck, Merck KGaA, Darmstadt, Germany, and Pfizer Inc. to develop a 500-plus gene liquid biopsy panel. They have a planned release for mid-2017.⁵⁴ This signals both diverse interest and the importance of partnering with pharmaceutical companies and industry. This partnership is likely to use gene testing for development of a type of companion diagnostics, in which biomarkers are elaborated and validated alongside drug development. In this scenario, diagnostic testing is paired with a clinical trial, and ultimately drug labeling, dictating the indications for use. This provides funding partnerships that accelerate the development of paired diagnostics and therapeutics.

Market research indicates the biomarkers sector has grown to \$39.4 billion in 2014. The market is expected to grow at a 5-year compounded annual growth rate of 13.8% from 2015 to 2020, increasing from \$50.6 billion in 2015 to \$96.6 billion in 2020.⁵⁵ The potential for growth needs to be reconciled with increased cost awareness in health care. Researchers and industry are going to have to prove savings of expanded testing either by reducing ineffective health care or improving health.

Conclusions

For those of us interested in biomarkers, the challenges as well as the benefits seem tremendous. To make the necessary advances in cancer treatment, a patient's disease must be monitored in an efficient and effective manner. There must be the ability to repeat sample and to identify biomarkers from those samples that are testable in real time. It is therefore the goal of biomarker research to monitor from a liquid biopsy taken from urine or saliva or blood in a noninvasive manner. The marker itself, whether protein or nucleic acid or CTC, must have sufficiently high sensitivity and specificity to predict the development of a particular form of disease. Moving our focus from the treatment of late-stage diseases to the monitoring and management of diseases caught at an early stage, will perhaps move medicine from a curative model to one of prevention. Harnessing the power of academia, industry, big pharma, government, social media, and their combined resources will prove instrumental in not only finding novel biomarkers but also unleashing their potential immediately instead of it taking 140 years, as it did with Bence Jones.

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Author Contributions

MJS and NRW conceived and designed the experiments and contributed to the writing of the manuscript. MJS analyzed the data and wrote the first draft of the manuscript. MJS, NRW, and MS agree with manuscript results and conclusions; jointly developed the structure and arguments for the paper; made critical revisions and approved final version; and reviewed and approved the final manuscript.

Disclosures and Ethics

As a requirement of publication, author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality, and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material.

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