

# Minireview

## Biomarkers: Delivering on the expectation of molecularly driven, quantitative health

Jennifer L Wilson<sup>1</sup> and Russ B Altman<sup>1,2</sup>

<sup>1</sup>Bioengineering Department, Stanford University, Stanford, CA 94305, USA; <sup>2</sup>Department of Genetics, Stanford University, Stanford, CA 94305, USA

Corresponding author: Jennifer L Wilson. Email: wilsonjl@stanford.edu

### Impact statement

Precision medicine evolved because of the understanding that human disease is molecularly driven and is highly variable across patients. This understanding has made biomarkers, a diverse class of biological measurements, more relevant for disease diagnosis, monitoring, and selection of treatment strategy. Biomarkers' impact on precision medicine can be seen in cancer, pharmacogenomics, and safety. The successes in these cases suggest many more applications for biomarkers and a greater impact for precision medicine across the spectrum of human disease. The authors assess the status of biomarker-guided medical practice by analyzing themes for biomarker discovery, reviewing the impact of these markers in the clinic, and highlight future and ongoing challenges for biomarker discovery. This work is timely and relevant, as the molecular, quantitative approach of precision medicine is spreading to many disease indications.

### Abstract

Biomarkers are the pillars of precision medicine and are delivering on expectations of molecular, quantitative health. These features have made clinical decisions more precise and personalized, but require a high bar for validation. Biomarkers have improved health outcomes in a few areas such as cancer, pharmacogenetics, and safety. Burgeoning big data research infrastructure, the internet of things, and increased patient participation will accelerate discovery in the many areas that have not yet realized the full potential of biomarkers for precision health. Here we review themes of biomarker discovery, current implementations of biomarkers for precision health, and future opportunities and challenges for biomarker discovery.

**Keywords:** Biomarkers, precision medicine, therapeutics, drug discovery, molecular, mechanisms

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### Introduction

Biomarkers in a general sense have long been implicated in diagnosing and treating disease; precision medicine has made this practice bigger, broader, and more specific. Strimbu and Tavel eloquently define biomarkers as “the most objective, quantifiable medical signs modern laboratory-measured science allows us to measure reproducibly.”<sup>1,2</sup> Clinicians have already used biological measurements, such as blood type<sup>1,2</sup> and blood pressure,<sup>1</sup> in clinical decision making. However, big data techniques

have processed and extracted a previously unprecedented scale for the precise measurement of biological features,<sup>3–6</sup> and researchers have used numerous techniques for biomarker discovery: metabolomics,<sup>7</sup> proteomics,<sup>8</sup> genomics,<sup>9,10</sup> epigenetics,<sup>11</sup> and lipidomics.<sup>12</sup> Coupled with the acknowledgement that the same disease can vary greatly across patients<sup>13,14</sup> and the ability to measure features at the patient level,<sup>5</sup> the definition of suitable biomarkers has evolved; the Food and Drug Administration (FDA) and National Institutes of Health (NIH) recently convened to

define and describe biomarkers to harmonize these efforts.<sup>15</sup> This evolution and the success of existing biomarkers has impacted our working definition of precision medicine. Precision medicine sets the expectation of quantitative, often molecular, biomarker measurements for management of disease.

However, creating personalized, mechanistic approaches to medicine is complicated. Biological pathways complicate biomarker identification and validation, rapidly evolving regulatory guidelines slow biomarker development, and limitations in data access and measurement techniques make biomarker discovery an incomplete search. Despite these challenges, biomarkers' utility for precision medicine has promoted the growth of research programs, development of technology, and application of new scientific approaches for advancing precision medicine. These features reflect on how biomarkers are delivering on the promises of precision medicine. We highlight themes for identifying biomarkers, where biomarkers are used clinically, and future directions for biomarker technology.

## Molecular biomarkers as the pillars of precision medicine

Molecular biomarkers are a manifestation of the shift in therapeutic development from "one-size fits all" to individualized, patient-matched healthcare.<sup>16</sup> In the former paradigm, small molecule compounds were screened for their effects on clinical outcomes at the population level. In the latter, patient conditions are viewed as a heterogeneous mix of individualized molecular abnormalities resulting in similar clinical symptoms.<sup>2,4</sup> In this personalized paradigm, therapeutic strategies are designed against known molecular features of a clinical outcome.<sup>1,2</sup> These molecular features are the core of personalized medicine and are intended to enable clinical decision making.

Quantitative, molecular phenotypes are becoming the mainstay of precision medicine. In this development context, the term biomarker refers to features such as gene variants, a circulating protein, or combinations of these features<sup>2</sup> that are expected to correlate with underlying biology and may be predictive. Clinically, these features can be prognostic, diagnostic, predictive, or response markers<sup>17</sup> and scientists use any and combinations of these categorical biomarkers to describe these aspects of disease. Recent work by the FDA and NIH provides specific definitions for diagnostic, monitoring, pharmacodynamics/response, predictive, prognostic, safety, and susceptibility/risk biomarkers, and reasonably likely surrogate and validated surrogate endpoints.<sup>15</sup> Here we examine high-level themes that motivate biomarker discovery.

### Single, biological features track disease at all stages

The search for informative biomarkers started with single, biological measurements that correlated with a clinical decision; these measurements signify a disease, track disease prognosis, or associate with a treatment response. In inflammatory bowel diseases (IBD), C-reactive protein

(CRP), fecal calprotectin, and fecal lactoferrin are evolving as markers for predicting response to therapy and differentiating between IBD disorders.<sup>18</sup> A collaboration between European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology have differentiated asthma into distinct endotypes, or pathological subtypes.<sup>19</sup> However, there have been mixed successes with applying biomarker-driven decisions to treatment. Omalizumab, an anti-IgE treatment, evolved as the first implementation of precision medicine for asthma patients.<sup>19,20</sup> Since Omalizumab, anti-IL-5-targeted treatment which selects patients based on blood or sputum eosinophil counts, has expanded the biomarker toolset for asthma patients.<sup>19,20</sup> These examples reflect trends towards large patient cohorts and the use of big data for characterizing disease. However, identifying these features is only the first step; using these markers clinically requires further validation and longitudinal investigations.

### Pathways underlie disease, and drugs function within these pathways

The pathways revolution in biomedical science acknowledges that disease results from the concerted effects of multiple genes and proteins instead of the classical perspective that disease is caused by single, driving mutations or proteins. Historically, drugs with clinically relevant outcomes have not had mechanistic links to underlying disease pathways.<sup>21,22</sup> Drugs were often approved ahead of complete biological and pharmacological understanding if and when a desired clinical outcome was possible.<sup>21</sup> Pathways have provided a construct for understanding the interconnected action of multiple genes and proteins, and have changed how scientists interpret disease and approach treatment.

This pathways perspective further motivated a molecular understanding of health and contributed to the search for molecular biomarkers. This type of understanding of lung and colorectal cancer motivated the repurposing of anti-ErbB targeted anti-cancer therapies across indications because these diseases share dysfunction in similar pathways. Similarly, mutations in the lamin A/C gene can give rise to cardiomyopathy, muscular dystrophy, lipodystrophy, and progeria.<sup>6</sup> Integrated network approaches leverage the interactions among genes and proteins to better understand disease; this type of approach specifically enhanced differences between basal and luminal breast cancer types. As more diseases are characterized by the underlying molecular abnormalities, treatment decisions will continue to move from the tissue of origin to the pathway of origin. Repurposing of drugs across indications will benefit pharmaceutical companies and could mitigate the expected loss of revenue associated with the notion that personalized therapies will suit smaller, more specific patient groups.

Pathways have already guided biomarker identification through synthetic lethality approaches, a popular technique for uncovering genetic vulnerabilities altering response to treatment.<sup>23,24</sup> These investigations screen combinations of gene knockouts without a mechanistic understanding of the relevant pathway. For instance, BRCA

mutations sensitizing patients to Poly(ADP-Ribose) Polymerase 1 (PARP) inhibitors is an example of a genetic liability altering treatment efficacy<sup>25</sup> discovered without interaction pathway information. However, having a complete understanding of a drug's pathway interactions could expedite the identification of sensitizing mutations, drug interactions, or the risks of drug combinations to guide biomarker discovery.

### Surrogate biomarkers could be the shortcut to the development pipeline

Clinical trials with surrogate biomarkers are a deviation from historical approaches where effects on clinical outcomes are required. Historically, drugs were approved based on their reduction in clinically derived symptoms of disease.<sup>21</sup> The AIDs epidemic in the 1990s promoted an accelerated approval path where drugs could reach the market for conditions where the clinically relevant outcome—such as mortality—required extremely long, and expensive clinical trials. In this accelerated scenario, drugs could receive approval using a reasonably likely surrogate marker—a measurement with a mechanistic/epidemiological connection to a clinical outcome<sup>15</sup>—or a validated surrogate endpoint—similar to a reasonably likely marker, but with clinical trial evidence to show connection to the clinical outcome.<sup>15</sup> In this context, many surrogate biomarkers manifested from improved understanding of disease mechanisms. Surrogacy trials emphasized these mechanistic hypotheses over clinical outcomes for the sake of efficiency.<sup>21</sup> Surrogate biomarkers and endpoints have aided in the design, and implementation of clinical trials, have made metrics for novel therapies more standardized and consistent, and expedited the discovery process by shortening trial time and requiring fewer patients.<sup>21</sup>

Unfortunately, the ability to validate surrogate biomarkers forestalls this promise.<sup>21,26</sup> The appeal of identifying surrogate markers is clear: with an adequate molecular measurement, it is possible to claim an effect on clinical outcomes without requiring the longitudinal study to reach these clinical endpoints. The Cardiac Arrhythmia Suppression Trial showed that reduction in ventricular arrhythmias was not a sufficient surrogate marker for death following myocardial infarction<sup>27,28</sup> and multiple clinical trials showed that reduction in brain amyloid and cerebrospinal fluid (CSF) phosphorylated tau were not correlated with clinical outcomes for Alzheimer's patients,<sup>29,30</sup> even though mechanistic hypotheses supported these ideas. Features that correlate with clinical outcome may not track the outcome under drug intervention,<sup>21</sup> and a thorough understanding of the disease pathways, and the pathways affected by the drug is needed to assess whether an intervention is having a clinically useful effect on the measured surrogate.<sup>21</sup> For these reasons, the process of validating a biomolecular measurement as a sufficient proxy for the disease-relevant clinical outcome has become arduous.

There are a handful of successfully validated surrogate markers, including cases for cardiovascular and cancer

drug development. Gefitinib, erlotinib, crizotinib, and certinib have used biomarker-based patient stratification to improve clinical trial outcomes and expedite therapeutic development<sup>25</sup> by better matching patients to efficacious treatments. For cardiovascular drugs, surrogate markers have helped to reduce the cost of therapeutic development; however, their discovery required post-market analysis. Randomized clinical trials of anti-hypertensives with distinct mechanisms saw reductions in stroke and coronary heart disease; the inclusion of distinct therapeutic interventions supports the notion that reductions in blood pressure are a sufficient surrogate for stroke and coronary artery disease clinical outcomes.<sup>31,32</sup> The blood pressure example is promising, but data from longitudinal, meta-analyses are not available for experimental new drugs.

Prevalence of surrogate biomarkers in clinical trials is increasing, but surrogate biomarkers are hardly mainstream due to the challenges of their discovery process. An analysis of clinical trials conducted during 2002–2009 showed that the use of biomarkers in clinical trials doubled from 5% of clinical trials in 2002 to 10% of clinical trials in 2009.<sup>33</sup> Antineoplastic agents had the highest representation of clinical trials using biomarkers at 37.1%; lipid modifying agents and diabetic agents represented 6.1% and 5.0% respectively of biomarker studies.<sup>33</sup> However, this analysis discovered that the number of late-stage clinical trials using biomarkers was relatively infrequent.<sup>33</sup>

Further characterization of surrogacy markers requires basic research of disease mechanisms coupled with epidemiological and longitudinal studies of patients. Fleming and Powers argue that a surrogate biomarker is sufficient when the causal disease pathways are known, when the marker's effect on the causal disease pathway is understood, and when an understanding of the intervention's "off-target" effects are known. Fleming and Powers also caution that the pursuit of biomarkers dilute efficacy signals because of the biomarker's distance from the original clinical outcomes.<sup>26</sup> We also lack an understanding of how these biomarkers distinguish spectrums, or degrees, of disease.<sup>34</sup> This level of understanding will require concerted effort from data scientists, clinicians, and statisticians.

### Biomarker-guided decisions in the clinic

Clinicians are already using biomarkers in clinical decision making. Cancer and drug safety are two areas where clinicians have used biomarker measurements for the implementation of precision medicine. The field of pharmacogenomics overlaps with these application areas, but expands the notion of biomarker-guided decisions to all drugs and uniquely emphasizes the role of genetic features. In addition to affecting clinical decision making, biomarkers have spurred innovation, and promoted the growth of research consortia for the sake of precision medicine discovery. While these are only a handful of examples, their success affirms the expectation that biomarkers will continue to influence precision medicine applications.

## Cancer as a paragon of precision medicine

Biomarkers have had a prominent impact on the implementation of precision medicine for cancer and are becoming a mainstay in the management of cancer patients therapy. Molecularly, cancer is a heterogeneous disease; only 10–30% of the same cancer type respond to the same drugs,<sup>35</sup> and further, distinct sub-clones cause differential drug sensitivity within a single patient.<sup>36,37</sup> In lung cancer, there is not a single treatment for all patients; instead, physicians treat patients based on EGFR or ALK expression status.<sup>25</sup> Additionally, mutational status of the prognostic biomarker, PIK3CA, can predict a patient's response to first-line therapies for HER2-positive breast cancers.<sup>38,39</sup> Using Trastuzumab and EGFR inhibitors to treat colorectal cancer depends on KRAS mutational status.<sup>26</sup> Molecular characterization of cancer also includes functional assays; checkpoint inhibitors, including Keytruda, are now prescribed for patients based on their DNA mismatch repair capacity in addition to measuring PD-L1 expression levels and non-synonymous mutation burden.<sup>40–42</sup> There are many cancers without sufficient biomarker definitions, meaning that the toolset of molecular features for clinical decision making is constantly changing.

The complexity of disease and the number of molecular entities contributing to pathology contribute to this continual evolution.<sup>6</sup> The heterogeneity in cancer confounds the ability to narrowly identify robust therapeutic targets<sup>4</sup>; on average, solid tumors carry 30–60 mutations, where lung cancers can carry upwards of 150 mutations.<sup>25</sup> Further, each subclass of lung cancer—adenocarcinoma, squamous cell carcinoma, and small cell lung carcinoma—had mutations in a few similar driver genes such as TP53, but the remaining top-ranked mutations differed across each of these subtypes.<sup>25</sup> Even as researchers measure all features associated with disease, it is difficult to separate correlative features from causative features.

Matching patients to therapy is most straightforward when the driving molecular feature, often a mutation, is also a drug target.<sup>25</sup> In these treatment contexts, having a molecular decision that defines a treatment strategy does not guarantee a better clinical outcome.<sup>26</sup> In the case of KRAS mutation status in advanced colorectal cancer, absence of mutated KRAS was associated with better response to Cetuximab but was not associated with overall survival in patients receiving the non-Cetuximab, standard of care.<sup>43</sup> The cancer precision medicine pipeline requires outcome-driven biomarkers in addition to molecular features that define therapeutic susceptibility.

## Biomarkers in safety

There are sufficient examples of how biomarkers better characterize drug safety. Human leukocyte antigen (HLA) variants help clinicians prevent hypersensitivity and adverse reactions to abacavir,<sup>44</sup> carbamazepine,<sup>45</sup> and allopurinol.<sup>46</sup> Pharmacogenomics has further characterized how HLAs, drug transporters, drug metabolizing enzymes<sup>47</sup> affect drug action. CYP2D6 affects metabolism of tricyclic antidepressants, and codeine; in the latter, codeine treatment can cause life-threatening effects if the

patient is a CYP2D6 hypermetabolizer.<sup>47</sup> Reduction in CYP2C19 function impairs patients' ability to process clopidogrel, an anti-coagulant, reducing therapeutic efficacy.<sup>48</sup> CYP2C9 and VKORC1 are genotyped prior to warfarin prescription because genetic variability affects the appropriate, effective dose.<sup>49</sup> The serum markers, cardiac troponin and natriuretic peptides have some predictive value for identifying cardio toxicity after anthracycline treatment.<sup>50</sup> These examples cement the utility of biomarkers for increasing drug safety.

Traditional development tools are not sufficient for predicting all sources of adverse drug events. Safety investigations have used chemo-informatics, systems biology, and *in vitro* and *in vivo* screening to discover the molecular underpinnings of toxicity and side effects.<sup>51</sup> Screening a drug *in vitro* against known modulators of adverse events is a relatively fast and inexpensive process for ruling out promiscuous candidates, compared to post-marketing surveillance.<sup>51</sup> But these binding screens are hardly exhaustive of all proteins modulating toxic side effects. Safety and efficacy are two of the top reasons that drugs fail to reach the market.<sup>52</sup> This suggests that a better understanding of biomarkers associated with toxic side effects could drastically improve drug discovery.

Systems pharmacology has started identifying the core proteins mediating adverse events.<sup>53</sup> A combined database (MetaADEDB) and drug side effect similarity inference method identified drug-binding partners mediating off-target effects for antiasthma and anti-depression medications.<sup>54,55</sup> Network modeling using protein-protein interactions uncovered mediators of drug-induced rhabdomyolysis,<sup>56</sup> drug-induced peripheral neuropathy,<sup>57</sup> Stevens-Johnson Syndrome,<sup>58</sup> and drug-induced liver injury.<sup>59</sup> A related meta-analysis of these adverse events further uncovered common protein mediators of these phenotypes and discovered that drug mode-of-action was responsible for these phenomena.<sup>60</sup> These emerging studies suggest a mutual maturation of understanding of adverse event mechanisms and discovery of applications for precision medicine.

## Pharmacogenomics as a source of gene–drug interactions

Remaining clinical examples of biomarkers have resulted from the growth of pharmacogenomics. While some cancer and safety applications overlap with this field, pharmacogenomics is precision medicine with an emphasis on genetic features and has applications to drug selection, prognosis, and safety. This sub discipline is focused on identifying genetic variants that affect drug response<sup>16</sup> and describing clinical decisions associated with these variants. Clinicians select doses of warfarin based on a patient's CYP2C9, CYP4F2, VKORC1 genotype, and the presence of the variant, rs12777823.<sup>49</sup> The Pharmacogenomics Knowledge Base (PharmGKB) and the Clinical Pharmacogenetics Implementation Consortium (CPIC)<sup>61</sup> currently catalogue similar recommendations for 36 compounds, and provide dosing guidelines for the administration of these compounds. Further examples include using IL28B variants for

predicting response to interferon- $\alpha$  treatment in hepatitis-C patients.<sup>62</sup>

A large pharmacogenomics community has blossomed around efforts to discover pharmacogenes, yet there are many unanswered questions. CPIC<sup>61</sup> catalogues specific, clinically relevant genes for treating patients, and PharmGKB curates studies investigating gene-drug relationships from basic science to clinical implementation.<sup>63</sup> Genome-wide association studies (GWAS) are a mainstay of PGx research, although, there are limited number of these studies compared to GWAS for diseases, and far fewer GWAS for understanding toxicity or adverse events.<sup>64</sup> Developing pharmacogenomics dosing guidelines will rely on investment in GWAS investigations. Additionally, patients often use multiple prescriptions simultaneously. With 42.6% of the elderly population taking >5 drugs,<sup>65</sup> pharmacogeneticists will need GWAS investigating drug combinations or computational methods to understand drug-gene-drug interactions. This suggests many opportunities for basic science projects in the discovery of pharmacogenes.

Many factors contribute to the momentum of Pharmacogenomics: The cost of DNA sequencing is decreasing suggesting the availability of patient DNA,<sup>6</sup> genetic biomarkers are attractive because they do not suffer from reverse causation,<sup>66</sup> and increasing patient participation in clinical research<sup>2</sup> has fueled repositories, such as the UK Biobank,<sup>67</sup> for bioinformaticians to search for new markers of genomic medicine. Electronic health records have contributed greatly to bioinformatics research and precision medicine<sup>6</sup>; yet, patient genetic data is not widely available. Although, some argue for preemptive testing,<sup>68</sup> this bottleneck is due to ethical and logistical hurdles.<sup>69</sup>

## Features affecting biomarker progress

The previous sections highlighted the motivations for biomarker discovery and how these biomarkers have enacted the expectation of a molecularly driven, quantitative approach to health in the clinic. This section considers the regulatory, commercial, and biomedical technologies that both accelerate and impede the development of precision medicine approaches. In these areas, there is an assumption that biomarkers can impact ongoing and future health challenges.

### Commercial pressures shape biomarker science

Precision medicine will continue to be disruptive, but application of biomarkers must also be cost-effective to ultimately be useful in the clinic.<sup>70</sup> Market segmentation can be a disincentive for pharmaceutical companies to design biomarker-based strategies. Pharmaceutical companies must balance the quest for new, more specific drugs at the expense of replacing broadly profitable, less specific compounds.<sup>6</sup> Related, pharmaceutical companies also must consider the costs of bringing therapies to market. A survey of clinical development success rates from 2006–2015 found that drugs with biomarkers were 25.9% likely to reach market compared to 8.4% of drugs without

biomarkers.<sup>52</sup> From a return-on-investment standpoint, a company is encouraged to pursue biomarker discovery if the cost of clinical development is to remain the same.

Diagnostic biomarker assays have another financial hurdle due to insurance infrastructure, and this has mixed effects on their development. Insurance companies reimburse diagnostic tests less frequently than treatments,<sup>71</sup> and are unlikely to cover the cost of a test unless approved by the FDA.<sup>34</sup> Further, evaluations of clinical benefit must be compared to the current standard of care to earn insurance coverage.<sup>34</sup> Insurance coverage does not include Florbetapir, an imaging agent for AD diagnosis, because there is no link between the imaging agent and better clinical outcome<sup>34</sup> and improved patient/family planning has not been sufficient for insurance companies to consider coverage.<sup>34</sup> To date, insurance companies have largely pushed the burden of demonstrating value for biomarker technologies to pharmaceutical companies.

Financial costs associated with assay development and regulatory approval could be further disincentives in biomarker science. For instance, although ELISA assays have the potential to be high-throughput, developing and verifying an ELISA-based test cost \$100,000–\$2 million to develop for each biomarker.<sup>71</sup> After approval, the process of assaying the patient may also be cost prohibitive. In the case of patients awaiting coronary artery bypass grafting, prioritization of patients based on estimated glomerular filtration rate (eGFR) was considered cost-effective.<sup>72</sup> A combined approach measuring eGFR and circulating CRP was less cost effective than non-biomarker guided prioritization due to the cost of the assay.<sup>72</sup> In a similar case, the cost of a CSF diagnostic assay altered the utility of diagnosing patients with this method.<sup>73</sup> More specifically, at an Alzheimer's disease (AD) prevalence rate of 9.1% and below, biomarker-based diagnosis was not cost-effective due to the costs associated with the CSF assay. At 15% prevalence, the CSF procedure was cost-effective because the benefits of treatment outweighed the costs of the assay and the costs of false-positive treatments.<sup>73</sup>

Economic analyses will be crucial for the translation of biomarker science. The parameters in a cost-effective analysis include the costs of measuring the biomarker, the sensitivity and specificity of the biomarker assay, the quality-adjusted life years gained from the intervention, the risk-benefit of the intervention.<sup>74</sup> A comparison of the use of modified transesophageal echocardiography (TEE) against manual palpitation for preventing post-operative stroke in patients undergoing cardiac surgery found that the TEE diagnostic was more cost effective than the manual technique.<sup>75</sup> Their results showed that modeling can quantify the costs associated with a diagnostic test, and that for some disease and patient population combinations, these tests are cost-effective relative to existing strategies.<sup>75</sup>

### A co-evolution with regulatory infrastructure

Regulatory infrastructure has had mixed effects on approved biomarker applications. In the US, success in biomarker applications has influenced the Critical Path Initiative at the FDA. This regulatory mission defines key

areas for the future of medicine, including innovation around biomarker technology and the use of 'omics measurements for precision health. Similar regulatory support lags in other countries (such as Japan)<sup>33</sup> and thus there has been less development in biomarker-driven studies in these locations. The US has the largest share of oncology studies due to the National Cancer Institute's (NCI) relatively large portion of the NIH budget. This spending trend combined with the biomarker-guided theme in oncology has made the US the leader in biomarker discovery. Regulation provides the motivation to invest in surrogate marker efficacy and relevancy. When using surrogate markers, the FDA requires sponsors to conduct post-marketing investigations to ensure that the surrogate markers affect the clinically relevant outcome.<sup>21</sup>

Further, the allure of accelerated drug approval for orphan diseases has altered biomarkers' role in therapeutic discovery. Some cancer drugs, such as ALK inhibitors have received accelerated status due to the small number of lung cancer patients with ALK mutations. Because biomarkers divide patients into subclasses within a disease, it becomes possible to define highly represented diseases as orphan using biomarker stratification. Due to the possible risks associated with accelerating a drug to market, Kesselheim *et al.* have argued that orphan designation should be withheld for any cancer with an ALK mutation below a certain threshold regardless of tissue of origin.<sup>76</sup>

As with any innovative technology, regulatory and industry scientists find insufficient or conflicting guidelines. FDA regulation in the US has already provided an infrastructure and incentives for innovation; however, regulation will continue to change as biomarker technologies evolve.

### **Biomarker "dark matter"**

Simply put, there is a lot we have not measured. Omics measurements, data storage, and clinical implementation are expensive and, thus, are restricted to a few geographic regions.<sup>77</sup> The socioeconomic nature of precision medicine research and implementation has created a gap in the patients able to receive personalized therapy.<sup>77</sup> Early applications of Warfarin dosing algorithms showed higher sensitivity in Caucasian and Asian populations due to the low representation of other ethnicities in study cohorts.<sup>78</sup> Many other study cohorts suffer the same bias and lack representative samples from which to draw broad conclusions. A systematic application of existing 'omics technology across patient populations could drastically increase our genomic coverage and confidence in selecting biomarkers.

We also know that most diseases are the culmination of more than genetic lesions, and we have not yet explored the complete landscape of features contributing to disease. Environmental, epigenetic, and lifestyle factors all contribute to disease,<sup>4</sup> yet are not always considered during big data, 'omics approaches. Michael Snyder's group is using biosensors to characterize the human physiome and have identified deviations and patterns indicative of Lyme disease and inflammatory responses.<sup>79</sup> Biomarker discovery will benefit from further characterization of the concerted

role of these features and systems biology techniques for integrating and dissecting numerous biomarker types.

While proteomics and metabolomics stand to be powerful, non-invasive biological measurements, technological hurdles limit the use of these technologies; namely an inability to measure the majority of biomolecules and difficulty in measuring disease-related molecules due to signal-to-noise challenges.<sup>71</sup> Metabolomics has identified amino acids that identify patients predisposed to diabetes, discovered the atherotoxin trimethylamine N-oxide, and identified harmful oncometabolites.<sup>80</sup> Studies of the cancer secretome have uncovered potentially useful biomarkers,<sup>81</sup> yet are not fully integrated into preclinical and clinical pipelines. Technological improvements in coverage of measurements will further cement the utility of these technologies for biomarker discovery.

Historical clinical trial approaches may be poorly suited to assess some biomarkers. Instead, basket trials, umbrella trials, and N-of-1 trials may be better suited to discovering molecular features governing response to treatment.<sup>82-84</sup> Seminal investigation into non-small cell lung carcinoma enrolled patients using a molecular classification instead of traditional histopathological classification. In the CUSTOM trial, they enrolled patients for a total of 15 study arms, demonstrating the utility of these basket trials for studying rare mutations.<sup>85</sup> The NCI MATCH trial is a further extension of this model and enrolls patients based on molecular profiling to identify responders.<sup>86</sup> Innovative clinical trial designs will continue to be important in identifying biomarkers with clinical utility.

### **Biomarkers beyond the bench**

Molecular measurements of disease are extending beyond clinical-grade assays.<sup>2</sup> Integrated technology and the internet of things (the ability of everyday devices to submit and receive data through integration with the internet) has promoted an explosion of patient-centered data, and opened a dearth of possibilities for tracking non-traditional biomarkers. Biosensors measuring the human physiome can monitor normal human patterns and identify deviations that are indicative of disease, such as Lyme disease and inflammatory response from skin temperature and heart-rate data.<sup>79</sup> Before wearables, measurement of these features occurred infrequently, making it difficult to identify a patient's baseline or normal amount of deviation.<sup>79</sup> Smartphone apps collecting data using the Patient Health Questionnaire-9 (PHQ-9) correlated scores with clinically administered surveys, although, the app detected higher levels of suicidal ideation.<sup>87</sup> However, smartphone apps do not produce research quality data,<sup>88</sup> and much of these data are not available to clinicians due to the intended market of health-related apps.

Wearables and personal measurement technologies are going to continue to mature and thus create more complex and detailed images of patients. Continuous monitoring will unlock previously uninformative biomarkers, and creation of new wearable measurement technologies, reporting apps, and integration software will aid in the identification and integration of non-traditional

biomarkers. Just as glucose meters and insulin pumps have made the patient immediately responsible for insulin treatment decisions, wearables will continue to empower the patient to make real-time treatment decisions.

## Opportunities and challenges for precision medicine discovery

Collins and Varmus articulated a promising future for precision medicine, predicting key innovation areas.<sup>2</sup> To date, the biomedical research community has identified numerous molecular measurements which characterize disease, inform treatment, and alter definitions of disease. They accurately predicted that cancer would become a flagship case for applying precision medicine and that the principles learned for these patients would be extended to patients suffering from many diseases. Cancer will continue to be a flagship application of precision medicine approaches, but many features will accelerate the expansion of precision medicine to other indications: development in big data infrastructure, greater patient participation, and the prevalence of wearables and the internet of things. This section investigates where these features are poised to answer precision medicine questions.

### Precision medicine's unfinished business

The low-hanging fruit in precision medicine lies where there is data. The cancer community has amassed large collections of 'omics data measuring disease in different tissues and under varying treatment regimens,<sup>89-91</sup> and some argue that genomic data collection has outpaced our ability to understand these results.<sup>92</sup> Bioinformatics and systems biology approaches stand to identify novel biomarkers and uncover areas where biomarkers are relevant across cancer indications. Pharmacogenomics will benefit from large repositories of patient genetic data such as the UK Biobank<sup>67</sup> and the 1000 Genomes Project.<sup>93</sup> Innovation in biomedical informatics, biostatistics, and computational modeling will continue to advance precision medicine for cancer.

Further, there are already numerous examples of leveraging patient sequencing databases for novel discovery. Researchers have conducted mortality investigations,<sup>94</sup> identified ethnic disparities in predisposition to diabetes,<sup>95</sup> and uncovered novel genetic associations with impaired lung function.<sup>96</sup> These data repositories will become valuable for identifying, tracking, and validating biomarkers as well.

### Current research ecosystem gives genetic biomarkers an advantage

Sequencing DNA is standardized, the technology is becoming pervasive, and there is sufficient regulatory support for benchmarking sequencing data and guidelines for identifying a genomic biomarker. Further, a patients' genome has applications beyond the initial motivation for sequencing and can be a stable source of decision making throughout the patients' medical history. The resounding challenge is that DNA is not always the complete biological answer.

However, when there is a mechanistic link, sequencing makes good sense as a starting point for biomarker discovery.

### Time is precision medicine's nemesis, technology can help

The hardest precision medicine challenges occur over long time scales. Discovering and validating surrogate biomarkers is a noble goal with enormous potential impact. However, for many diseases, validating these markers requires significant understanding of disease and therapeutic interventions over time. AD is currently untreatable disease because we lack a full understanding of disease pathology, let alone the effects of a drug intervention.<sup>21,73</sup> Preventative treatments for AD are desirable because advanced disease is so aggressive. But without a clinical trial outcome to measure, the effects of preventative intervention are unmeasurable.<sup>34</sup> Imaging techniques are informative but do not reflect the underlying causes of the disease.<sup>73</sup> The first stages of AD biomarker discovery requires further clinical investigations and natural history studies.<sup>34</sup>

The AD case is representative of multiple diseases that will benefit from longitudinal and epidemiological studies before these indications benefit from the expediency of surrogacy-guided trials. Metabolomics and smartphone apps will play a role in these long-term studies because smartphones are pervasive, apps are relatively easy to deploy and because both techniques are non-invasive. Importantly, non-invasive techniques stand to passively collect data. Large, passively collected data could provide novel insights where it is unclear which measurements and at what time point are relevant to disease progression. Mendelian randomization, which identified aldehyde dehydrogenase as a risk factor for myocardial infarction in Japanese men,<sup>97</sup> could aid in the discovery of surrogate markers, but this process requires a sufficient genetic proxy for uncovering risk factors.<sup>98</sup>

### Precision medicine's underachieved value

Precision medicine has yet to reach a stage of universal clinical utility.<sup>92</sup> The largest challenge, and occasionally, largest cost in bringing precision medicine decisions to the clinic is interpretability,<sup>77</sup> and achieving this interpretability is complex.<sup>92</sup> For instance, many early efforts using 'omics data promoted the ability to find genes correlated with disease outcome; later researchers discovered that these sets were no better than random gene sets at predicting disease outcome.<sup>99</sup> Robustness is required to improve interpretation and thus, researchers have switched to more robust predictors and demonstrated their utility in stratifying cancer patients.<sup>100,101</sup>

However, the Institute of Medicine has made recommendations to achieve this interpretability based on the assumption that precision medicine will provide clinical value.<sup>92</sup> These recommendations focus on creating centralized, national resources for collecting and sharing electronic health records and promoting fair and equitable access to biomarker tests and data. A related recommendation calls

for flexible regulation to address concerns with current smartphone apps: apps that collect passive medical data could be subject to privacy and liability concerns.<sup>102</sup> Further, because regulation discourages app companies from using health app data to make medical decisions, it is possible that new regulation could release a new wealth of useful biomarker data. The convergence of these ecosystem innovations will accelerate discovery and validation of biomarkers and spur adaption of precision medicine to many indications.

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#### DECLARATION OF CONFLICTS OF INTEREST

RBA is the founder and a stockholder in Personalis and a stockholder in 23andme; he declares no conflict of interest.

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