Biomarkers in Pharmaceutical Research

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BACKGROUND: Biomarkers are important tools in drug development and are used throughout pharmaceutical research.

CONTENT: This review focuses on molecular biomarkers in drug development. It contains sections on how biomarkers are used to assess target engagement, pharmacodynamics, safety, and proof-of-concept. It also covers the use of biomarkers as surrogate end points and patient selection/companion diagnostics and provides insights into clinical biomarker discovery and biomarker development/validation with regulatory implications. To survey biomarkers used in drug development—acknowledging that many pharmaceutical development biomarkers are not published—we performed a focused PubMed search employing “biomarker” and the names of the largest pharmaceutical companies as keywords and filtering on clinical trials and publications in the last 10 years. This yielded almost 500 entries, the majority of which included disease-related (approximately 60%) or prognostic/predictive (approximately 20%) biomarkers. A notable portion (approximately 8%) included HER2 (human epidermal growth factor receptor 2) testing, highlighting the utility of biomarkers for patient selection. The remaining publications included target engagement, safety, and drug metabolism biomarkers. Oncology, cardiovascular disease, and osteoporosis were the areas with the most citations, followed by diabetes and Alzheimer disease.

SUMMARY: Judicious biomarker use can improve pharmaceutical development efficiency by helping to select patients most appropriate for treatment using a given mechanism, optimize dose selection, and provide earlier confidence in accelerating or discontinuing compounds in clinical development. Optimal application of biomarker technology requires understanding of candidate drug pharmacology, detailed modeling of biomarker readouts relative to pharmacokinetics, rigorous validation and qualification of biomarker assays, and creative application of these elements to drug development problems.

The Biomarkers Definitions Working Group, composed of members from the US Food and Drug Administration (FDA),4 NIH, academia, and industry, defines a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” This definition includes not only molecular biomarkers measured in the clinical laboratory, but also imaging technologies [i.e., x-rays, MRI, and computerized axial tomography (CAT), and positron emission tomography (PET) scans] and other physiological measurements such as blood pressure. This review covers only molecular biomarkers.

Drug development is costly and inefficient. Fewer than 12% of novel drug candidates that entered clinical evaluation from the 1990s to the 2010s received marketing approval, with safety and efficacy being the major reasons for failure issues (1, 2). Although the number of new molecular entities and therapeutic biologics annually approved remains at a plateau of approximately 20–30, the number of new FDA-approved drugs per billion US dollars of research and development spending in the drug industry has decreased by half approximately every 9 years since 1950, in inflation-adjusted terms (2, 3). The FDA recognized this unsustainable inefficiency in 2004, prompting the agency to launch the Critical Path Initiative to “drive innovation in the scientific processes through which medical products are developed, evaluated, and manufactured.” In the first Critical Path Initiative report, “Innovation/Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products” (4), the importance of biomarkers in improving drug development efficiency was emphasized. Since then, there has been a surge in the use of biomarkers in all

4 Nonstandard abbreviations: FDA, US Food and Drug Administration; CAT, computerized axial tomography; PET, positron emission tomography; TE, target engagement; HER2, human epidermal growth factor receptor 2; PD, pharmacodynamics; ROC, proof of concept; PK, pharmacokinetics; BTK, Bruton’s tyrosine kinase; DDP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; TK, toxicokinetic; CRP, C-reactive protein; HCV, hepatitis C; IUO, investigational use only; miRNA, microRNA; lncRNA, long noncoding RNA; NSCLC, Non–small cell lung cancer; EGFR, epidermal growth factor receptor; PCa3, prostate cancer antigen 3; C3L4, cytotoxic T-lymphocyte antigen 4; PD-1, programmed death 1; PD-1L, programmed death-ligand 1; T cell 2, IL-13, interleukin-13.
phases of drug development. The main phases of drug discovery and development are defined in Table 1, and biomarker types are defined in Table 2.

A PubMed search was performed employing “biomarker” and the names of the largest pharmaceutical companies as keywords and filtering on clinical trials and publication in the last 10 years. This yielded almost 500 entries. The majority of them involved disease biomarkers (approximately 60%) or prognostic/predictive (approximately 20%) biomarkers. The remaining included biomarkers of target engagement (TE), safety, and drug metabolism. Interestingly, a notable proportion (approximately 8%) was related to human epidermal growth factor receptor 2 (HER2). HER2 testing is used to identify patients with invasive breast and gastric cancer who are likely to benefit from treatment with herceptin (5). HercepTest™, the first HER2 test approved in 1998, is based on immunohistochemistry measurements and is considered to be the first approved in vitro companion diagnostic device. Today there are 8 approved devices for HER2 testing. In terms of citation number, oncology was the leading therapeutic area, followed by cardiovascular disease and osteoporosis. Diabetes and Alzheimer disease also engendered many citations.

This review initially addresses applications of biomarkers in clinical development, such as in TE, pharmacodynamics (PD), proof-of-concept (POC), safety, surrogate end points for regulatory approval, and patient selection/companion diagnostics. It then covers clinical biomarker discovery using different technological approaches, and ends with a general overview of the biomarker process and its regulatory implications.

**TE and PD Biomarkers**

TE biomarkers involve assays that directly measure the fraction of a target bound (occupied) by a drug under specific conditions. They are extraordinarily valuable in
drug development because they directly link pharmacokinetics (PK) (drug exposure) and resulting PD and efficacy. They also allow more rapid and informed program termination if required occupancies cannot be achieved with safe doses, or insufficient PD is observed despite high TE. Unfortunately, assessment of TE in clinical studies is not always attainable because of difficulties in accessing tissues in which drug targets are expressed and engaged. Hence, most TE assays involve either (a) accessing tissues in which drug targets are expressed and studies is not always attainable because of difficulties in high TE. Unfortunately, assessment of TE in clinical studies is not always attainable because of difficulties in accessing tissues in which drug targets are expressed and engaged. Hence, most TE assays involve either (a) accessing tissues in which drug targets are expressed and studies is not always attainable because of difficulties in high TE. Unfortunately, assessment of TE in clinical studies is not always attainable because of difficulties in accessing tissues in which drug targets are expressed and engaged. Hence, most TE assays involve either (a) accessing tissues in which drug targets are expressed and studies (single-photon emission CT) imaging of radiopharmaceuticals in situ (beyond the scope of this review), (b) plasma enzymatic activities if the target is an enzyme in circulation, or (c) molecules such as covalent inhibitors or monoclonal antibodies having sufficiently low apparent off-rates to allow sample handling and analysis without drug dissociation. The last is exemplified by a fluorescent protein Western blot developed to measure the TE of ibritinib, a covalent Bruton’s tyrosine kinase (BTK) inhibitor for hematologic malignancies (6). The assay uses a fluorescently labeled, second covalent BTK antagonist as an affinity probe. Because ibritinib and the affinity probe bind covalently, their associations with BTK persist over long handling and storage time, and after target denaturation. After mixing the probe with a sample from treated patients, the latter is lysed, denatured, and resolved using SDS-PAGE. A fluorescence reader is then used to evaluate TE, with higher ibritinib TE causing lower fluorescence of the BTK band on the blot. This assay both helped define preclinical in vivo exposure–occupancy relationships to better select initial clinical dosing and helped refine and verify clinical dose vs receptor occupancy predictions (6, 7).

When binding kinetics preclude clinical TE assays, an analyte immediately downstream of the target is measured as a TE proxy (i.e., “proximal PD”). Occasionally, a development program is availed of both. This is exemplified in the clinical development of sitagliptin, an inhibitor of dipeptidyl peptidase-4 (DPP-4) (8). DPP-4 cleaves and inactivates glucagon-like peptide-1 (GLP-1), a gut hormone that regulates blood glucose. In sitagliptin development, ex vivo plasma DPP-4 activity and plasma GLP-1 concentrations served as TE and proximal PD markers, respectively. These markers were well characterized in preclinical species and implemented in the translational strategy of sitagliptin. Specifically, preclinical experiments demonstrated that 80% inhibition of DPP-4 activity was associated with maximal lowering of glucose concentrations, which also correlated with an increase in plasma GLP-1 concentration. PK-PD modelling revealed that the EC80 (80% maximal effective concentration) of plasma DPP-4 inhibition corresponded to a plasma sitagliptin concentration of approximately 100 nmol/L. It was also determined that a single dose of 100 mg/kg provided DPP-4 inhibition (>80%) for 24 h. The ability to use these biomarkers to explicitly model the PK-PD relationship from dose to exposure to TE to proximal PD, and hence to distal PD (serum glucose and insulin) provided great confidence in the mechanism of action and the dose ranging, allowing elimination of a standard phase II POC study and greatly accelerating eventual approval of the compound. This was possible only through thorough preclinical biomarker characterization and subsequent aggressive translation to the clinic (8). Table 3 summarizes different types of biomarkers used in the development of sitagliptin. In addition, Table 4 lists selected examples of biomarkers in pharmaceutical research.

**SAFETY BIOMARKERS**

Safety biomarkers play a critical role in drug development and have been employed for decades, including well-characterized tests of organ function and/or injury for the

**Table 2. Biomarker types and definitions.**

<table>
<thead>
<tr>
<th>Biomarker type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target engagement</td>
<td>Fraction of target binding sites occupied by a drug</td>
</tr>
<tr>
<td>Pharmacodynamics</td>
<td>Drug effects on the human body to assess whether the downstream pathway or biological process regulated by a drug target is perturbed upon drug administration. These could be proximal or distal to the target. An ideal PD marker should show dose or exposure responsiveness and some correlation with efficacy. PD biomarkers are critical to establishing PK-PD models to help define safe and effective doses.</td>
</tr>
<tr>
<td>Disease activity</td>
<td>Effects of a drug on a particular disease, which occur late in the pathophysiologic cascade and are linked to clinical benefit. Also referred as POC biomarkers. These are also useful for PK-PD modeling.</td>
</tr>
<tr>
<td>Surrogate endpoint</td>
<td>Substitution for a clinical endpoint and prediction of clinical benefit with certainty. In some cases, such as with HIV viral load and hemoglobin A1c, these may be used as phase III endpoints to support marketing approval.</td>
</tr>
</tbody>
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Such TK modeling using creatine kinase as a marker of skeletal muscle toxicity was used by Cubist Pharmaceuticals to develop a nonmyotoxic dosing regimen for daptomycin, salvaging it from oblivion and providing physicians with a key weapon against resistant gram-positive bacteria (27).

### Biomarkers for POC and Beyond

The term “proof of concept” has been defined by the Pharmaceutical Research and Manufacturers of America as “the earliest point in the drug development process at which the weight of evidence suggests that it is “reasonably likely” that the key attributes for success are present and the key causes of failure are absent” (28). Practically, it is the point at which investment in large and costly dose finding and subsequent phase III studies is justified. Insufficient efficacy underlies the majority of failures in late-stage drug development, and using biomarkers to judge the probability of success is becoming increasingly important (1). Biomarkers offer the promise to conduct smaller and shorter clinical trials before performing larger, more expensive trials with conventional end points. To achieve POC most efficiently, the use of disease biomarkers and surrogate end points along with PD and safety markers is almost mandatory, as was the case in sitagliptin development (Table 3), for which the hemoglobin A1c served as the former.

### Table 3. Examples of biomarker types or clinical trial end points and their relationship to drug efficacy.

<table>
<thead>
<tr>
<th>Biomarker or clinical trial end point</th>
<th>Item measured</th>
<th>Example from sitagliptin</th>
<th>Degree of confidence on efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE</td>
<td>Fraction of drug target bound by drug</td>
<td>Plasma DPP-4 activity</td>
<td>+a</td>
</tr>
<tr>
<td>Proximal PD biomarker</td>
<td>Substrate or other molecule immediately downstream of target</td>
<td>Plasma GLP-1 (substrate)</td>
<td>+a</td>
</tr>
<tr>
<td>Distal PD biomarker</td>
<td>Further downstream biological effect</td>
<td>Plasma glucose, insulin, glucagon</td>
<td>++</td>
</tr>
<tr>
<td>Surrogate end point</td>
<td>Biological effect intimately associated with clinical outcome</td>
<td>Hemoglobin A1c</td>
<td>++++b</td>
</tr>
<tr>
<td>Clinical outcome</td>
<td>Mortality, morbidity, symptoms, quality of life</td>
<td>Myocardial infarction (MI), stroke, death from MI</td>
<td>++++</td>
</tr>
</tbody>
</table>

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a Although TE and proximal PD biomarkers may contribute similarly to confidence regarding potential efficacy, formal TE assays allow explicit understanding of the in vivo relationship between pharmacokinetics/exposure and PD. More commonly, a TE biomarker cannot be developed and proximal PD must be relied upon; however, this demands an assumption that some other pathway parallel to that of the target is not acting in vivo.

b Surrogate end points may be used to obtain regulatory approval, as with hemoglobin A1c, for diabetes, LDL cholesterol for secondary coronary prevention, and viral load for HIV drugs. However, occasionally these may be mechanism dependent and may be questioned for drugs using novel mechanisms and yet affecting the same biomarker (surrogate end point). This is exemplified by the ezetimibe IMPROVE-IT trial, its rationale and its implications (D’Nicolantonio et al. (9); Packard and Stezhka (10)).

Thus typically associated with stopping drug candidates with organ toxicities, safety biomarkers can also be modeled along with PK in toxicokinetic (TK) modeling to determine the relationship between the systemic exposure of a compound and its toxicity in experimental animals in a variety of dosing regimens. The optimal dosing regimen in animal studies can be used to guide the dose selection in human studies. Sometimes TK modeling can also rescue an apparently unsatisfactory drug candidate and improve the success rate in drug development.
tion, may be used alone or with other end points to monitor disease activity in many inflammatory diseases and allow rapid estimation of success of therapy (29). In the case of rheumatoid arthritis, the degree of inflammation reduction determines the extent of symptom relief and slowing of progression, although existing skeletal deformity is not generally reversible. Accordingly, CRP reduction in a small study of short-course infliximab contributed to rapid advancement of this compound, and the anti-TNF (tumor necrosis factor) mechanism more generally, to larger studies and eventual approval for rheumatoid arthritis (30). In contrast, the lack of established disease activity markers for chronic neurodegenerative diseases, such as Alzheimer and Parkinson diseases, precludes efficacy estimation in small studies and has led to many high-profile failures in the late stage of clinical development—such that several pharmaceutical efforts in this area have been abandoned (1, 31).

Disease burden biomarkers allow rapid clinical development, perhaps most prominently in infectious disease. Recent successes in HIV and hepatitis C (HCV) therapies directly result from availability of facile tools to monitor therapeutic effects on viral load. Criteria for viral load reduction derived from large clinical experience allows prediction of longer-term clinical outcomes, including cure (32, 33). In oncology, this role has classically been filled by imaging; however, laboratory tumor markers whose levels correlate with the degree of tumor burden may become available. Recent advances in molecular diagnostics with droplet digital PCR and next generation sequencing (NGS) may make it possible to follow tumor burden in real time through the assessment of mutation burden in cell-free DNA from patients on therapy (34).
Surrogate End Points

Surrogate end points are defined as laboratory measurements or physical signs that are used in therapeutic trials as substitutes for clinically meaningful end points that are a direct measure of how a patient feels, functions, or survives and are expected to predict the effect of the therapy (35). The main advantage of using surrogate end points during drug development is the ability to obtain accelerated approval through the FDA (36). There is a high bar for molecular biomarkers to attain surrogate end point status and only a few have done so (37). In general, 3 primary criteria must be met for surrogacy: (a) biological plausibility of the biomarker–outcome relationship; (b) high prognostic performance for the clinical outcome; and (c) clinical trials—based evidence of a correlation between treatment effect on the biomarker and on the patient outcome. Even these are not universally accepted and have generated some controversy (38). The most commonly used molecular surrogate end points are cholesterol for cardiovascular end points, hemoglobin A1c for diabetes end points, and viral load for HIV and HCV end points. Numerous drugs for coronary heart disease, diabetes, HIV, and HCV were developed using these end points to show clinical benefit and ultimately obtain regulatory approval (39).

In oncology, it has become commonplace to use progression-free survival as a surrogate end point for overall survival (40). Although in solid tumors this end point is based on imaging criteria, in leukemia progression-free survival uses minimal residual disease detection by molecular methods. The BCR-ABL transcript level illustrates the evolution and use of biomarkers to obtain approval of a novel therapy. Blood BCR-ABL transcript level, a marker of disease burden for chronic myeloid leukemia, was an exploratory end point in the phase III trial of imatinib. As clinical follow-up progressed, it became clear that BCR-ABL transcript level at 1 year of therapy could predict clinical outcomes even after 7 years, validating it as a surrogate end point (41). These data were used as a basis for obtaining approval for nilotinib, the follow-on to imatinib, after just 1 year of patient follow-up in a trial using BCR-ABL transcript level as the surrogate end point, leading to a significant acceleration of drug development (42, 43). BCR-ABL transcript level testing exemplifies the evolution of new surrogate end points from exploratory studies that generate a signal suggesting clinical utility to validation in prospective studies.

Patient Selection and Companion Diagnostics

In oncology, the discovery of oncogenic somatic mutations that generate gene products that can specifically be targeted by a drug has revolutionized the drug development of new treatments for patients with cancer. Because of the specificity of drug action, patients are selected for therapy on the basis of the molecular subtype of the cancer. The prospective matching of a drug with a patient harboring cancer with defined molecular characteristics requires the simultaneous development of drug and diagnostic to ensure the highest level of safety and efficacy (44). The diagnostic test paired with this type of targeted drug is called a “companion diagnostic” (45). The approval of both device and drug should ideally occur simultaneously for patients to benefit from therapy. This has been challenging because it requires coordination between different arms of the FDA, the Center for Devices and Radiological Health, Center for Drug Evaluation Research, and/or Center for Biologics Evaluation and Research. The complexity of codevelopment is also exacerbated by the fact that the device and drug are often developed by different companies, requiring a high level of coordination between the two. However, the FDA and the industry have made substantial progress on this front with several recent successful examples listed here (46).

A drug targeting a mutant protein, as above, provides the most straightforward context for a companion diagnostic, for which the need to match patients to the therapy is obvious and is integral to development from the start. For instance, vemurafenib, an inhibitor of the V600E mutant BRAF kinase in melanoma, required an assay to identify patients with melanoma harboring the B-Raf proto-oncogene, serine/threonine kinase (BRAF) V600E mutation starting from the initial clinical trial. In this case, a robust investigational use only (IUO) device suitable for registration with the FDA’s Center for Devices and Radiological Health was first developed. Second, the IUO assay was used for recruiting patients into the trial. Finally, data obtained from IUO testing showed a tight correlation between BRAF V600E status and vemurafenib response. These steps demonstrated the clinical utility of the BRAF mutation test, thus supporting the filing of the premarket approval application, and vemurafenib’s risk–benefit profile simultaneously supported the new drug application to the FDA. The drug and the diagnostic were marketed at the same time to ensure access of the therapy to eligible patients (47).

Clinical Biomarker Discovery

In general, 2 approaches are used in clinical biomarker discovery: an unbiased approach without a prespecified hypothesis and a targeted approach that tests a prespecified

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5 Human genes: BRAF, B-Raf proto-oncogene, serine/threonine kinase; ADCY9, adenylate cyclase 9; ERBB2, erb-b2 receptor tyrosine kinase 2; ZEB2, zinc finger E-box binding homeobox 2.
fied hypothesis. In the former, a range of omics profiling technologies is generally used to compare at least 2 different populations. Two key factors are carefully considered in study design. One factor is having well-characterized patient populations to which the gold standard for clinical diagnosis has been applied, and the second is a balanced design minimizing the number of uncontrolled covariates (e.g., age, sex, ethnic origin, environmental exposures, and sometimes geography). Also, sample collection, processing, and storage should be well standardized and well controlled to minimize preanalytical variability. In the following sections, we summarize the unbiased and targeted approaches, as well as technologies, with case studies of applications in clinical biomarker discovery.

Genomics for Biomarker Discovery

Genomic biomarkers measure changes in the quantity or nature of DNA or RNA. DNA-based biomarkers include copy number variation, mutations (including polymorphisms, somatic mutations, and other genetic variants), and epigenetic modifications. RNA expression-based biomarkers include quantification of mRNA, microRNA (miRNA), and long noncoding RNA (lncRNA) expression.

Biomarker discovery based on alterations in DNA typically aims to link individual single nucleotide polymorphisms or copy number variations to certain patient characteristics across a population. Multiple large-scale array-based profiling technologies can be used to conduct these studies. One example is the recent identification of genomic determinants of cardiovascular effects of the cholesteryl ester transfer protein inhibitor dalcetrapib (48). Tardif and colleagues conducted a genome-wide association study in the phase 3 clinical trial dal-OUTCOMES (efficacy and safety of dalcetrapib in patients with recent acute coronary syndrome) and a targeted genotyping in the dal-PLAQUE-2 [dalcetrapib and plaque imaging in 2 vascular beds (a study of the effect of dalcetrapib on atherosclerotic disease in patients with coronary artery disease)] imaging trial. One single nucleotide polymorphism, rs1967309 in the adenylate cyclase 9 (ADCY9) gene, correlated with cardiovascular events in the dalcetrapib arm ($P = 2.41 \times 10^{-8}$) but not the placebo arm, suggesting that polymorphisms in the ADCY9 gene may influence dalcetrapib’s effects on cardiovascular events and that rs1967309 or a related analyte may be a useful tool for tailoring cholesteryl ester transfer protein–based therapy.

Another type of DNA-based biomarker measures epigenetic DNA modifications such as methylation, changes in which are frequent in a diverse range of diseases. Non–small cell lung cancers (NSCLC) have multiple distinct subtypes with somewhat differing prognoses. Specifically, patients with epithelial-like tumors have greater sensitivity to inhibitors of the epidermal growth factor receptor (EGFR) pathway than patients with mesenchymal-like tumors. Using genome-wide methylation profiling and methylation-specific PCR analyses, Walter and colleagues identified patterns of DNA methylation in epithelial–mesenchymal transition–related genes, such as erb-b2 receptor tyrosine kinase 2 (ERBB2) and zinc finger E-box binding homeobox 2 (ZEB2), which can divide NSCLCs into 2 phenotypically distinct subtypes of tumors of potential therapeutic relevance (49).

Microarray-based technologies have also been applied to profile mRNA, miRNA, and lncRNA expression. For example, MacIsaac and colleagues identified a 256-gene signature in pretreatment whole blood that is associated with 14-week clinical response in infliximab-treated rheumatoid arthritis patients (50). miRNAs regulate gene expression posttranscriptionally by either causing degradation of specific mRNA or blockade of their translation. Some of them are expressed in a tissue- and disease-specific manner. miRNAs are very stable in plasma and serum, making them potentially facile analytes for the diagnosis and prognosis of human diseases (51). Using the nCounter miRNA expression and qPCR (quantitative real-time PCR) analyses, Kumar and colleagues identified a 7-miRNA signature in human plasma that may distinguish patients with Alzheimer disease from healthy controls (52).

In contrast to miRNAs, lncRNAs locate within the intergenic stretches or overlapping antisense transcripts of protein coding genes that regulate gene expression and cellular functions. These transcripts possess a low, but tissue-specific and timely, restricted expression manner. Recently, they have emerged as promising biomarkers. A successful example is prostate cancer antigen 3 (PCA3/ DD3). Bussemakers and colleagues initially identified DD3 as an alternatively spliced lncRNA specifically overexpressed in >95% of primary prostate tumors (53). Subsequently, urinary PCA3/DD3 was examined in multiple clinical studies with favorable results. In addition, the pivotal study of 466 men, with 21.9% identified as having prostate cancer, demonstrated that a PCA3 score cutoff of 25 yielded 77.5% diagnostic sensitivity, 57.1% diagnostic specificity, and negative and positive predictive values of 90% and 33.6%, respectively (54), leading to the FDA approved urine-based molecular diagnostic test, the PROGENSA® PCA3 assay, to help determine need for repeat biopsies in men with previous negative prostate biopsies. Furthermore, in the REDUCE (Reduction by Dutasteride of Prostate Cancer Events) trial of dutasteride for chemoprevention of prostate cancer, the PCA3 assay outperformed prostate specific antigen for cancer detection and improved the diag-
nostic accuracy when combined with prostate specific antigen and other clinical variables (55).

In addition to array-based technologies, massively parallel or NGS has emerged as a rapid and high-throughput technology for single or multigene sequencing. NGS provides an unprecedented view into complex DNA and RNA samples, reporting on mutation, copy number variance, modification, and expression. One example is whole-exome sequencing. Antibodies against immune checkpoint inhibitors such as anti–cytotoxic T-lymphocyte antigen 4 (CTLA-4) and anti–programmed death 1 (PD-1) antibodies have demonstrated significant clinical benefits in a subgroup of patients with metastatic melanoma (56–58). Using whole-exome sequencing on tumor tissue, Snyder and colleagues discovered a neoantigen (tetrapeptide) signature that was specifically present in tumors with a sustained response to CTLA-4 blockade, and then further validated the neoantigen signature in an independent cohort (59). Thus, examining exomes of melanoma patients could inform responses to the anti–CTLA-4 blockade therapy in disease management decisions.

In contrast to the array-based or NGS approach, a targeted genomics approach can also be used in biomarker discovery. For example, Owczarczyk and colleagues discovered a combination mRNA biomarker, IgκFCRL5κ, in whole blood to identify about 20% of patients with active rheumatoid arthritis who are unlikely to gain substantial clinical benefit from anti-CD2–B-cell depletion therapy (60).

Proteomics for Biomarker Discovery

A powerful but challenging approach for protein biomarker discovery is label-free differential mass spectrometry. Hendrickson and colleagues used a high-resolution differential mass spectrometry platform to profile cerebrospinal fluid from postmortem diagnosed patients with Alzheimer disease and nondemented individuals and identified multiple peptides exhibiting statistically significant differences between the groups (61). Two peptides, SME-1 and SME-2, were selected and confirmed in an independent longitudinal cohort. Moreover, levels of SME-1 and SME-2 in Alzheimer disease were estimated to decrease 10.9% and 6.9% per year, respectively, suggesting that these 2 peptides could be used as quantitative tools to assess the effects of disease-modifying agents in drug development.

In addition to open-ended profiling, focused protein assays have been used in biomarker discovery. For instance, using mass spectrometry–based multiple reaction monitoring assays, Wildsmith and colleagues identified chitinase-3–like protein 1 in cerebrospinal fluid as a longitudinal dynamic biomarker in Alzheimer disease (62). Additionally, using an aptamer-based focused profiling platform, Zhao and colleagues identified a 5-protein classifier in plasma to identify patients with Alzheimer disease (63). As discussed above, objective disease activity biomarkers are generally lacking for neurodegenerative diseases. This leads to signal dilution by enrollment of misdiagnosed patients into clinical studies, and precludes early POC efforts. The above efforts reflect attempts to rectify this problem.

Metabolomics and Lipidomics for Biomarker Discovery

Metabolites are low molecular weight compounds such as sugars, amino acids, organic acids, and nucleotides. The approaches for metabolite and lipid biomarker discovery are very similar to LC-MS–based proteomics profiling. For instance, Mapstone and colleagues identified and validated a 10-lipid classifier in plasma that demonstrated over 90% diagnostic accuracy in predicting phe-noconversion of healthy controls to either amnestic mild cognitive impairment or Alzheimer disease within 2–3 years (64).

Immunohistochemistry and Flow Cytometry for Biomarker Discovery

Immunohistochemistry is often used to uncover whether a specific analyte that is intimately associated with a drug target can serve as a predictive marker for clinical response. For example, in a phase 1 clinical study for the anti-PD-1 monoclonal antibody nivolumab, Topalian and colleagues performed an immunohistochemistry analysis for programmed death–ligand 1 (PD-L1) on pretreatment tumor samples from 42 patients with melanoma, NSCLC, colorectal cancer, renal-cell cancer, and prostate cancer (65). None of the 17 patients with PD-L1–negative tumors had an objective response. In contrast, 9 of 25 patients (36%) with PD-L1–positive tumors had an objective response (P = 0.006). Thus, PD-L1 expression on the surface of tumor cells could potentially be used to identify patients for anti-PD-1 antibody therapy and guide disease management decisions in the clinic.

Flow cytometry allows sorting of cells from complex biological matrices and measurement of analytes on the surface or inside of cells. This technology enables measurement of receptor occupancy/TE by monoclonal antibody therapeutics on specific cell subpopulations. Using a flow cytometric assay in peripheral blood mononuclear cells, Brahmer and colleagues observed mean occupancy of >70% of PD-1 molecules on circulating CD3+ T cells lasting at least 2 months following nivolumab infusion across a large dose range (66). Using the same assay, Topalian and colleagues observed the median PD-1–receptor occupancy by nivolumab as 64%–70%
on peripheral blood mononuclear cells from 65 patients with melanoma who were treated with 1 cycle of nivolumab at a dose of 0.1–10.0 mg/kg (65). In both cases, this facilitated dose modeling.

Despite this striking progress in multidimensional biomarker discovery technology, most promising biomarker candidates still prove useless for clinical prediction and decision-making, as observed in a prominent commentary (67). Indeed, many of the above biomarker discovery efforts have not yet led to practical tests. Of the many reasons for this, among the most common are insufficient predictive power despite sometimes striking statistical significance arising from a large number of patients and/or analytes studied, and poor extrapolation from the clinical trial setting to broad clinical practice. This is a particular problem in inflammatory diseases such as asthma and lupus that have poorly understood pathobiology and lack the highly distorted cell-autonomous signaling pathways and/or the somatic mutations often present in cancer. Nevertheless, examples of promising predictive biomarkers in this area are emerging—such as serum periostin in asthma (68, 69). Disease activity biomarkers can guide patient selection in clinical trials and practice, particularly for heterogeneous syndromes such as asthma that encompass differing pathobiologic phenotypes requiring tailored therapy (70). Periostin’s relevance arises from a rough dichotomization of asthma into helper T-cell 2 (Th2)-high and Th2-low phenotypes: the former involving exaggerated airway interleukin-13 (IL-13) function, increased serum IgE, peripheral and airway eosinophilia, and increased exhaled nitric oxide (70). Alone, the preceding elements are suboptimal biomarkers because of weak prediction and/or inconvenience (e.g., the sputum induction required for airway eosinophil counts). Airway mRNA profiling studies, however, revealed a set of mRNAs, including periostin mRNA, preferentially upregulated in patients with asthma (71). Moreover, airway periostin mRNA strongly correlated with Th2-high asthma (72). Critically important to development of a facile biomarker, serum periostin protein concentrations recapitulated much of the information encoded in airway periostin mRNA; furthermore, serum periostin concentrations were more stable over time than either blood eosinophils or exhaled nitric oxide (68). Th2-high asthma refractory to inhaled glucocorticoids represents a significant unmet medical need for which Th2-specific therapeutics are in development. In phase II studies of lebrikizumab, an experimental anti–IL-13 monoclonal antibody targeting those patients, high serum periostin strongly correlated with good drug response, potentially overcoming the problem of poor predictive power and suggesting potential utility as a patient selection biomarker. Phase III studies are required to determine whether this promise can be extrapolated to broader clinical use (73).

Overview of Biomarker Process and Regulatory Considerations

Biomarkers follow varied paths into clinical trials for pharmaceutical development. Some, like TE and PD biomarkers, transition from preclinical pharmaceutical experimentation because they are tightly linked to the drug being developed. Others may be incorporated into clinical protocols from the literature or from common clinical practice. In either case, before implementation in clinical studies, candidate biomarkers are subjected to validation and qualification. Analytical validation ensures adequate assay performance in relation to the questions addressed in the clinical study (fit-for-purpose). Biomarker qualification entails collecting sufficient evidence of the relationship between a biomarker with the relevant biological processes and clinical end points. Although the terms validation and qualification are sometimes used interchangeably, the distinction between them is important and each fits into the drug development process differently. Biomarker assay validation is performed in a laboratory equipped to support clinical studies following regulatory guidelines, whereas biomarker qualification is achieved by the execution of, sometime multiple, clinical studies.

In contrast to biomarkers in the clinical laboratory, which are subject to CLIA guidelines for analytical validation, biomarkers used in pharmaceutical research had not been under clear regulatory guidance until recently. In contrast, the validation and implementation of PK assays, have long been under regulatory oversight (74). The latest draft FDA guideline for industry was released in September 2013 (75), which for the first time included general guidelines for biomarkers and diagnostics kits. In this document, it was acknowledged that “biomarkers can be used for a wide variety of purposes during drug development; therefore, a fit-for-purpose approach should be used when evaluating the extent of method validation that is appropriate.” It is stated that biomarker method validation should aim to address the same performance characteristics as required in method validation for PK assays, and that the approach used for PK assays should be the starting point for validation of biomarker assays—although the FDA acknowledges that direct application of PK guidelines to biomarker assays is probably not possible. The FDA also suggested that the level of analytical validation of an assay should depend on the purpose of the biomarker data. For instance, an assay should be fully validated when the data are used for go/no go decisions. It should be up to
the sponsor to decide what level of validation needs to be applied on a fit-for-purpose approach. The FDA also made recommendations for diagnostic kits used to measure biomarkers in clinical trials, such as demonstration of the suitability of diagnostic kits for use in PK or PD studies and assessment of the performance of diagnostic kits in the facility conducting the sample analysis. Though these are rather general guidelines, their inclusion of biomarkers implies that such assays and laboratories supporting them for clinical trials may soon come under regulatory oversight.

Conclusions

Biomarkers have shown great promise in making the drug development process more efficient and have become an integral part of pharmaceutical research. This is reflected by the large number of clinical studies using biomarkers to support decision-making. It is expected that many of these biomarkers will ultimately transition into the clinical laboratory as the codevelopment of drugs with their companion diagnostics becomes more commonplace in the industry.

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