Over 2000 years ago, Hippocrates and the ancient Greeks believed that humors (bodily fluids) determined an individual’s behavior and disposition (On the Nature of Man, attributed to Hippocrates, 4th century BC). Although subsequent advances in genetics and biochemistry have disproved this, the powerful idea that our blood and tissues may hold the keys to health and disease has taken root. What is probably the first modern biomarker report was published in 1848 by the English physician and chemist Henry Bence Jones, who discovered that immunoglobulin light chains could be detected in the urine of about three-quarters of patients with multiple myeloma (Bence Jones, 1848). This remarkable discovery and many others like it have formed the basis of a modern field known as Precision Medicine, whose core mission is to diagnose illness faster and more reliably and to treat patients more effectively. In a personalized way, this means recommending an intervention for some diagnosed patients and recommending against the same treatment in others, thereby increasing the quality of health care across a population and the efficacy of a health system. In many ways, Precision Medicine can be an answer to the dual-mandate of many health systems to reduce cost and morbidity/mortality simultaneously. Within the past decade, this field has become one of the fastest-growing areas of modern medicine. Precision Medicine has instilled new vigor and enthusiasm in drug development, with its promise of shortening the time-lines from discovery to approval and improving the probabilities of success for late-stage development. Biomarkers are now being studied at each stage of this process: from preclinical discovery and early experimental medicine studies to the development of drug-device “companion” diagnostics included in filed regulatory packages. Rapidly developing technologies and the declining cost of high-throughput testing has created the possibility to acquire large datasets across multiple analytical platforms and shed light on new biology. As with many paradigm-shifting ideas, Precision Medicine is not without challenges. Choosing fit-for-purpose methods, managing extremely large data sets, and integrating findings from multiple platforms (“Big Data”) are some of the largest hurdles to be overcome.

When biomarker testing becomes a part of standard-of-care diagnosis and treatment selection, there is also the issue of cost. Although the price of individual tests, especially whole-exome and whole-genome sequencing, has been consistently declining, biomarker testing must be executed efficiently so as not to place too high a burden on overall cost of healthcare. While scientists play a critical role in developing simple and high-quality assays, the process of adoption of testing depends on a complex network of key stakeholders including physicians, patient advocates and powerful patients, policy makers, and payers. Despite these and numerous other challenges, many biomarkers are now included in standard-of-care diagnostics and therapies, and the evidence is growing for improved patient care across systems, aided by Precision Medicine initiatives and adoption.
Role of Biomarkers and Precision Medicine in Modern Development

The Twentieth Century witnessed a great increase in average life expectancy in developed countries, from 45 to about 77 years (Kola and Landis, 2004). This change is likely a consequence of a number of improvements in these nations in healthcare, standard of living, and socio-economic environment. Despite a growing number of patients with chronic illnesses and increased unmet medical need in many therapeutic areas (some of which are a consequence of longer life) and a slowly expanding range of beneficial pharmaceutical interventions, the development of new drugs today is facing unprecedented challenges.

Drug development, on a per-approved-drug basis, is becoming increasingly expensive, despite the somewhat higher success rates seen in the biotechnology industry (Evens, 2016). The failure rate remains relatively high, even in fast-growing therapeutic areas such as immuno-oncology (Hollingsworth and Biankin, 2015). Prediction of success in drug development is based on complex mathematical models aimed at simulating risks and determining accurate net present value (NPV) of products, loosely defined as a product’s expected value adjusted for the probability of success (Trusheim et al, 2011; Svennebring and Wikberg, 2013). Implementation of Precision Medicine and other translational paradigms is expected to improve the NPV of drug pipelines in at least two important ways. First, by obtaining more information earlier in the development process, when trials are smaller and less expensive, the probability of success can be estimated more accurately, and low-probability programs may be discontinued. Secondly, by directing the drugs to patients most likely to benefit and less likely to experience toxicity, the efficiency of healthcare delivery is enhanced. A more efficacious drug may realize a higher price-point at launch (Mullane et al, 2014).

One of the largest drivers of cost in drug development is the size and duration of large, multi-site, late-stage clinical trials. Reducing size and duration of individual trials can be achieved early on by implementing a deeper study of the pharmacodynamics and biological changes occurring while the trials are still small. A better understanding of the impact of intervention on disease pathology allows for more accurate power calculations and appropriate sizing for larger trials. Early development trials may not only be reduced in duration, but may also have an increased predictive power in regard to the outcome of longer late-stage trials driven by clinical and outcome efficacy endpoints. Integration of the proof of biology approaches in early clinical development is likely to significantly improve the accuracy of predictions related to the endpoints required for registration. Selecting “surrogate” and biomarker endpoints strongly correlated to the clinical outcomes and registration endpoints is not a trivial task and requires not only deep understanding of the biology and molecular underpinnings of the drug activity, but also precise selection of assays. The assays’ performance specifications must be aligned with the requirements of conceptual hypothesis and inherent biological variability. Identifying and validating a “fit-for-purpose” assay is essential for the successful execution of information-rich, biomarker-driven early stage clinical trials and to inform decisions at the program and also the drug-class level. Although the predictive power can never be absolute, the impact on the development pipelines and landscape in general is a more realistic assessment of the probability of success in executing much larger, outcomes-driven studies. Increasing precision in the evaluation of the net present value of a pipeline product can be an invaluable asset in the highly unpredictable environment of drug development. Precision Medicine can thus be seen as a platform for a multi-pronged approach leading to reduction in attrition rates, cost of products to a healthcare system, and the cost of drug development in general. Improved prioritization strategies should be driven by the scientific understanding of the drug candidate’s mechanism of action in a context of a complex biological system (and also mechanisms of resistance in those systems), allowing for in-depth understanding of the requisite paths for successful clinical development and marketing.

Implementation of Biomarkers in and Beyond Clinical Trials

It is widely recognized that biomarker discovery ideally commences well before the initiation of a first-in-human development program in order to impact downstream development and marketing decisions in a meaningful way. To that end, a close collaboration between clinical and research scientists must be encouraged and fostered. Clinical scientists must be able to suggest experiments and biomarker validation tests to be performed in Research, and likewise, research scientists must be able to understand the limitations and challenges facing clinical development programs, including ease of execution of laboratory protocols, problems in obtaining tissues and fluids, and costs. The physical and geographic separation of clinical and research divisions seen in many drug development organizations is another barrier to effective collaborative discussion.

The key to identifying novel biomarkers and validating them in the context of a specific therapeutic intervention lies in early disease model work. Today, preclinical screening of potential drug candidates often includes biomarker assessments in addition to primary pharmacological characterization and efficacy determinations. The interdisciplinary approach to biomarker discovery in preclinical models of disease is largely enabled by novel technologies. The ability to test for multiple analytes in a relatively small volume of blood or tissue empowers high-throughput screens for discovery of biomarkers of interest and subsequent validation. In addition to the enhanced power of in vitro assays, significantly improved
animal models springing from multiple transgenic and chimeric platforms are of special importance. More accurate and powerfully predictive “humanized” murine models (Sheer and Wilson, 2015; Sanmamed et al, 2016), based on either genetic modifications of mouse genome representing a subset of genetically-engineered mouse models (Sheet at al, 2013; Sheer and Wolf, 2014) or engraftment of human cells or tissues in a rodent system, are a cornerstone of pharmacologic inquiry. Such models include patient-derived xenograft (PDX) cancer-bearing mice (Cassidy et al, 2015; Cho et al, 2016) and also the transplantation of human hematopoietic stem cells to generate “avatar” murine models (Goldman et al, 1998, Ito et al, 2002), etc. These models are widely used in a number of fields, especially in oncology, immunology, and infectious disease. Evaluation of biomarkers in a “humanized” in vivo preclinical setting is a crucial advancement that enables implementation of relatively mature biomarker strategies earlier in clinical development.

Accessibility of human tissue for research has also been a major driver in the processes of target validation and biomarker identification. An important goal of human bio-specimen collection in translational studies is to demonstrate a correlation between biomarker levels and disease onset or severity in a patient population of interest. The ability to reliably detect differential expression of specific biomarkers in the affected tissue and to correlate their expression or activity of specific molecular and cellular pathways to clinical presentation and outcomes not only enhances confidence in pursuing biomarker development strategies, but paves the way to a more precise implementation. Moreover, further biomarker validation in relatively small cohorts of patients either in the absence of treatment (such as in longitudinal natural history studies or cross-sectional disease-profiling studies) or with administration of minimal, sub-therapeutic drug levels (so-called “phase-zero” studies) is becoming more common.

One area that warrants continued attention is the translation of biomarker work and assays from the research into the clinical development setting. Rather than simply incorporate measurements of a suspected biomarker levels or activity across studies in the development program, one must ensure that the studies are adequately powered for assessment of the biomarker’s performance; thus, the performance characteristics of the assays and what constitutes meaningful change must be carefully elucidated.

It is also important to differentiate between prognostic and predictive potential of specific biomarker candidates and to allow for power calculations based on such assumptions. While prognostic biomarkers may inform on likelihood of disease progression irrespective of treatment, predictive biomarkers can be employed to estimate likelihood of response to a specific therapeutic modality. Prognostic markers, although informative and widely utilized by the

---

**Figure 1.** The value of the Precision Medicine approach may be modeled according to the two largest critical inputs: (1) the increase in efficacy, measured in QALYs, in the biomarker-positive population, and (2) the percent of the overall disease population identified by the diagnostic marker. Markers which identify larger populations and which add significantly to efficacy favor the development of a drug-diagnostic pair. This is true from both the pharmaceutical company and the physician/payer perspectives.
treating physicians to determine type of care a patient requires, do not directly identify a particularly effective therapeutic approach. For example, LDL cholesterol is an excellent prognostic biomarker of risk of heart disease and although it does indicate a possible need for a change in dietary and lifestyle habits, it does not suggest any specific treatment that is likely to benefit an individual patient. Understanding the molecular and genetic basis of cholesterol elevation in an individual patient, as well as possible safety implications for different therapeutics is key to selecting an appropriate therapy. Specific, predictive, well-validated biomarkers for hypercholesterolemia treatments will be tremendously useful.

Drug development, by its natural course, gravitates toward predictive biomarkers as tools for asset prioritization, drug repositioning/repurposing, and patient stratification. The identification of a reliable biomarker (or a group of biomarkers) characterized by sufficient sensitivity to predict clinical outcome due to intervention at the individual patient level is a precious asset. The ultimate goal of the Precision Medicine approach is to offer the most efficacious treatment for each individual patient, while limiting risks and exposure to ineffective treatments that are not likely to significantly alter the course of the disease, and may provide nothing but toxicity. The regulatory path for implementing this approach in the USA and certain other countries requires the registration of companion diagnostics that enable patient selection based on the results of testing for a predictive biomarker (or more frequently a panel of biomarkers) and which have demonstrated relatively high positive- and negative-predictive value (PPV and NPV, respectively). Ideally, the development and regulatory approval for a companion diagnostic occurs within the same time-frame as the drug development itself so as not to delay drug launch, as demonstrated in the recent example of a PD-L1 immunohistochemistry assay (Dolled-Filhart et al, 2015) which is now FDA-approved as a companion diagnostic for Keytruda® (an anti-PD-1 therapeutic monoclonal antibody). The time component of this process is critical and incorporating biomarker-related testing early in research and development is imperative so that biomarker and patient-selection validation may be seamlessly incorporated into late-stage development. A parallel process of clinical biomarker validation and development, coinciding with the development of the companion drug, enables better planning for marketing strategies and enhances the likelihood of a successful drug-diagnostic launch.

Another feature of potential biomarkers and companion diagnostics that is often overlooked at the earlier stages of development is the feasibility of testing. Unlike the preclinical environment, which is often conducive to invasive sampling of abundant amounts of biomaterials and very high sample quality, the clinical environment is much more difficult. Invasive sampling is often contra-indicated in individual patients, and each subject must furthermore consent to such sampling, with the absolute right to refuse any procedure. Too often, samples painstakingly obtained are later lost due to errors such as inattentive handling, shipping delays, or simply failure to follow procedures. Non-invasive sampling has great potential to enhance adherence to protocols in clinical trials and to improve implementation in the context of routine clinical care. For example, the successful introduction of “liquid tumor biopsies” that allow for analysis of representative circulating tumor cells (CTC) or circulating tumor-derived nucleic acids would allow for serial sampling and better monitoring of genetic and functional changes in the tumor (Ignatiadis et al, 2015). The development of non-invasive biomarker procedures is not only strongly preferred from an ethical standpoint, but it is also likely to reduce overall cost burden, leading to a better testing rationale from a payer perspective. Despite a trend toward reducing cost per single analytical test, the Precision Medicine approach may be seen to increase healthcare costs, since the cost of testing each member of the population is added to the cost of therapy. However, the increase in efficiency of delivery, in which patients are getting the best potential treatment and avoiding ineffective ones, will offset the cost of implementation of reasonably-priced diagnostics. It is important that payers see that the long-term benefit is likely to vastly outweigh the initial cost increase. Maintaining focus on the vision for overall improvements that are to be gained by Precision Medicine implementation is crucial. Supporting data must be demonstrated to payers and national governments, as the overall costs of healthcare increase due to multiple factors including an aging population, a higher rate of chronic diseases, and increased drug and procedure costs.

Role of Technology
The ability to generate huge volumes of data in a single experiment is a relatively new phenomenon in biology, leading to serious challenges in analyzing and integrating large data sets. Controlling for multiplicity has become a central issue of biostatistics. Techniques in tiered analysis and rigorous statistical methods for eliminating or minimizing bias have only partially solved the problem. Even the most rigorously analyzed data can be over-fitted in models of complex systems or be misinterpreted in the context of clinical relevance. The advent of inexpensive and virtually unlimited cloud-based computing has enabled the storage and computation of very large datasets, but interpretation must remain a scientist’s primary concern. One key is to identify and refine methods for data integration and modeling that prevent over-fitting of data and empower correlation with clinical outcomes. Such methods are important to ascertain whether systems biology and other “big data” approaches may be considered capable of producing conclusive results for a given biological question. While these approaches are necessary and powerful as tools of discovery, they must be followed by validation in biologically relevant settings. One may argue that the final proof must be obtained in a functional (in vitro or preferably in vivo) assay before initiation of clinical research studies for further validation. This goal may be achieved by approaching biomarker discovery and validation as an iterative process that should be allowed to veer from clinical data to mechanistic preclinical testing and back until an equilibrium is reached.
Although the project did not reveal such secrets as were speculated by some in the media, the reverberations of this achievement and follow-on work are felt daily in the scientific community. A number of genetic tests and screens are routinely used as part of the standard of care and for patient stratification; for example, EGFR mutational status in certain tumor types, HLA-B*5701 testing to predict abacavir hypersensitivity, cell-free screening, and fetal tissue karyotyping in prenatal diagnostics. With the cost of DNA sequencing plummeting over the last decade and continuing to fall, sequencing entire genomes or exomes of large population cohorts has become feasible and affordable.

New bioinformatics tools have enabled analyses of the sequencing results in a timeframe short enough to inform clinical decisions and treatment options. Integration of the genetic results from individual patients and affected families may shed light on genetic factors relevant for development and progression of disease, but the complexity of genetic and epigenetic interactions requires careful examination. The key step in bringing genetics to the clinic in a truly meaningful way will be development of associated high-throughput and high-quality assays for the validation of predictions from genetic findings. These may include biochemical validation of protein-protein interactions, cell-based in vitro assays, transgenic animals “humanized” on multiple loci of relevance, and others. Genetics is an extremely powerful tool in the modern age, but it cannot stand alone. It must be successfully integrated along with other testing and hypothesis evaluation into well-constructed clinical studies.

Future Directions

The progress Precision Medicine has made over the last few decades in increasing the efficiency of healthcare delivery has been astounding. A few critical next steps will define the near-term future of the field. Tools and strategies for scientific discovery must be wisely chosen. Technologies and markers must be validated and implemented well into clinical trials. Expensive tests must not be favored over less expensive ones unless justified by long-term and population-wide outcomes. Precision Medicine has the capability to address some of the major problems faced by insurers and governments around the world who want to reduce healthcare costs without sacrificing quality. Indeed, the Precision Medicine Initiative introduced by the United States in January 2015 may be a harbinger of substantial change in U.S. healthcare policy that will enable wider implementation of Precision Medicine in a more impactful manner. Working toward the goal of more efficacy, less toxicity of interventions across populations, while embracing practical implementation and marketing solutions, must be a priority for scientists everywhere.

Ana Kostic, Ph.D. is a clinical scientist with experience in drug development, biomarkers, and patient selection. She is currently Associate Director of Precision Medicine at Regeneron Pharmaceuticals. Dr. Kostic received her training in molecular and cell biology at Columbia University prior to joining Regeneron in 2008.

Robert Philips, Ph.D. is a clinical scientist with many years’ experience in biomarkers, patient selection, and development of drug-linked diagnostics. He is currently Head of Precision Medicine at Regeneron Pharmaceuticals in New York. Dr. Phillips received his training in molecular biology at Princeton University prior to joining Merck & Co. and, later, Novartis Oncology before accepting his position with Regeneron in 2011.

References

1. Hippocrates (alternatively attributed to his pupil Polybius). On the Nature of Man approximately 4th century BC.
10. Sannamniet MF, Chester C, Melero I, Kohrt H. Defining the optimal murine models to investigate immune checkpoint blockers and their combination with other immunotherapies. 6 Feb 23. pii: mwd041. [Epub ahead of print]