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COMMENTARY

Diabetes and Biomarkers

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Abstract

Biomarkers play an integral part in conducting clinical trials and treating patients. In most instances, they help medical practitioners, researchers, and regulatory officials make well-informed, scientifically sound decisions. However, in clinical studies, there is often uncertainty in how much weight to place on biomarker results versus clinical outcomes. This uncertainty emanates from opposing goals of the drug approval process. On one hand, the process must ensure that all therapeutics tested are safe and that the benefits outweigh the risks. On the other hand, the process should allow therapies to be accessible to patients as quickly as reasonably possible. Judicious use of biomarkers in the drug development process can bring these goals into alignment. More efficient discovery and use of biomarkers in the development of antidiabetes drugs will depend on advancing our understanding of the pathogenesis of diabetes and especially its macrovascular complications.

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At a joint meeting of the Food and Drug Administration (FDA) Endocrinologic and Metabolic Drugs Advisory Committee and the Drug Safety and Risk Management Advisory Committee on the drug Avandia, a panelist stated that "the road to regulatory hell is paved with surrogates."[1] While this statement may accurately reflect the state of affairs in clinical trials for new antidiabetes agents, hopefully the sentiment will be short-lived.

The Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[2] Diabetes clinical trials have long relied on biomarkers for proving efficacy of study drugs. Although hemoglobin A1c (HbA1c) was first separated from other forms of hemoglobin by Huisman and colleagues[3] in 1958 using a *chromatographic column*, its use for monitoring the degree of control of glucose metabolism in diabetes patients was first proposed in 1976 by Koenig and coworkers.[4] Managing diabetes based on HbA1c levels as a reflection of glycemic control was validated in 1990 by Larsen and associates.[5] Since then, regulatory agencies have widely embraced HbA1c as the biomarker of choice for proving efficacy in clinical trials for antidiabetes drugs. For example, the FDA's Guidance for Industry states that "for purposes of drug approval and labeling, final demonstration of efficacy should be based on reduction in HbA1c (i.e., HbA1c is the primary endpoint of choice, albeit a surrogate), which will support an indication of glycemic control."[6]

It is clear that HbA1c is far from a perfect biomarker. For example, levels of postprandial glucose,[7] as well as acute fluctuations in glucose levels,[8] may be better predictors of cardiovascular disease in diabetes compared

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Abbreviations: (CAST) Cardiac Arrhythmia Suppression Trial, (FDA) Food and Drug Administration, (GWA) genome-wide association, (HbA1c) hemoglobin A1c, (HDL) high-density lipoprotein, (LDL) low-density lipoprotein, (SNP) single-nucleotide polymorphism

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with levels of HbA1c. However, the FDA guidance states that new drugs whose mechanism of action is restricted to effects on postprandial glucose should be tested in dose-finding, proof-of-principle, short-term oral glucose studies. Such demonstrations of pharmacodynamic activity are not sufficient evidence of efficacy for new drug application approval, because the link between modifying effects on postprandial glucose excursions to clinical outcomes is not sufficiently strong enough to consider the use of this pharmacodynamic endpoint as a surrogate for efficacy.[6] Clearly, the relationship between hyperglycemia and clinical outcomes must be better understood in order for regulatory guidance to evolve from its current state, in which approval is based on a drug's ability to lower HbA1c without inducing a signal for excess cardiovascular risk (discussed here).

It has been assumed that tight blood glucose control would lead to lower rates of both microvascular and macrovascular complications of diabetes. This assumption was in large part due to the well-established fact that, as HbA1c rises, the risk of cardiovascular and all-cause mortality also rises.[9] Although the U.K. Prospective Diabetes Study documented the role of glycemic control in lessening microvascular disease in type 2 diabetes mellitus (T2DM),[10] a reduction in myocardial infarction and all-cause mortality was seen only in the intensive treatment group after long-term followup.[11] Because of the high rates of cardiovascular disease in those with T2DM, other studies were undertaken to explore the relationship between glycemic control and cardiovascular risk. Unfortunately, these trials, such as the Veteran's Affairs Diabetes Trial of Glycemic Control and Complications in Diabetes Mellitus Type 2, the Action to Control Cardiovascular Risk in Diabetes trial, and the Action in Diabetes and Vascular Disease: Preterax and Dimicron MR Controlled Evaluation trial, largely failed to show a beneficial effect of intensive versus conventional diabetes therapy on cardiovascular outcomes in subjects with long-standing diabetes.[12–14]

The lack of clarity around the relationship between glycemic control, macrovascular events, and survival in diabetes plays a central role in the firestorm of controversy concerning the possible relationship between rosiglitazone (Avandia) use and increased cardiovascular risk. Rosiglitazone is associated with statistically significant increases in total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and decreases in free fatty acids; however, in 1999 when rosiglitazone was approved for use in the United States, most thought that lowering HbA1c would be protective against cardiovascular disease and trump effects on the lipid profile, or at least be partially offset with the addition of concomitant statin therapy.

The relationship between diabetes treatment, HbA1c, lipid levels, cardiac outcomes, and survival very likely exceeds the complexity level that our current knowledge base allows us to comprehend. As more metabolic syndrome features are added into the equation, the level of complexity increases. Simplistic assumptions about the fidelity with which a biomarker reports on a disease process are sometimes incorrect and typically incomplete. In fact, biomarkers may fail to be reliable indicators of a biological process or therapeutic intervention for a number of reasons.[15] Chief among these is that a drug may exert unanticipated effects through other known or unknown biological pathways than those measured by the biomarker. The Cardiac Arrhythmia Suppression Trial (CAST) serves as a vivid example of this concept. Because ventricular arrhythmia is known to be associated with an increased risk for cardiovascular death, it was reasonable to hypothesize that suppression of such arrhythmias after myocardial infarction would reduce mortality. However, the results from the CAST showed that successful suppression of ventricular arrhythmias (in this case, ventricular ectopy was the biomarker) with encainide, flecainide, and moricizine was associated with an increased risk of death.[16] Similarly, it is useful to revisit the spectacular phase III failure of torcetrapib. Torcetrapib is a cholesteryl ester transfer protein inhibitor and raises HDL levels. The ILLUMINATE study combined torcetrapib with atorvastatin; the decreases in LDL combined with increases in HDL were expected to revolutionize therapy for hyperlipidemia. The study was successful from a biomarker standpoint: a significant increase in HDL cholesterol (72%) and a decrease in LDL cholesterol (25%) were seen after 12 months of torcetrapib therapy.[17] The biomarker results from the ILLUMINATE study should have predicted clinical success. Unfortunately, however, the trial was terminated after only 1.5 years (median) of treatment due to a higher mortality rate in the torcetrapib + atorvastatin arm compared with the atorvastatin-only group.[17] Off-target effects of torcetrapib on blood pressure and the renin–angiotensin–aldosterone system may be part of the explanation, though it seems likely that assumptions about HDL, LDL, and cardiovascular disease are flawed.

The uncertainty around the reliability of HbA1c as the pivotal biomarker to assess the efficacy of antidiabetes drug candidates has resulted in a new regulatory guidance by both the FDA and the European Medicines

Agency.[18,19] These mandate that sponsors demonstrate acceptable cardiovascular risk by meta-analysis and/or a large outcomes-based clinical trial. This directive is understandable from a regulatory perspective, but an unintended consequence may be that fewer diabetes drugs will be developed due to the substantial increase in development costs associated with the regulatory guidance.[20,21] Given the alarming rate of increase in the global incidence of diabetes, barriers to drug development in this area can ill be afforded from a public health perspective. The way forward is clearly to identify biomarkers that serve as reliable surrogates for clinical outcomes in diabetes rather than simply glycemic control. Because cardiovascular disease accounts for such a large proportion of morbidity and mortality in diabetes, useful biomarkers will need to predict outcomes such as ischemic cardiac disease, heart failure, and stroke.

The discovery of cardiovascular biomarkers has progressed significantly as our understanding of the pathogenesis of ischemic cardiovascular disease expands. The Framingham Study established the role of family history, gender, age, and smoking, as well as crude biomarkers such as blood pressure and lipid levels, as risk factors for cardiovascular disease. Today, we struggle to understand the pathogenetic progression from plaque to unstable plaque to plaque rupture to thrombosis, ischemia, and necrosis, as these events relate to endothelial activation, oxidative stress, hemostasis, and immune/inflammatory cascades. Given the complexity of the disease process, it is unsurprising that no single biomarker (e.g., C-reactive protein) has yielded more than incremental advantage over traditional measures of risk. The appreciation of the role of lipoprotein-associated phospholipase A_2 in modulating plaque vulnerability represents further progress along this incremental spectrum of advances.[22]

In diabetes, further complexity is added by the common coincidence of metabolic syndrome. The components of metabolic syndrome, including insulin resistance, atherogenic dyslipidemia, hypertension, and a proinflammatory and prothrombotic state all contribute to cardiovascular risk. The secretory activity of adipose tissue, including production of "adipokines," such as leptin, tumor necrosis factor alpha, interleukin-6, and adiponectin, likely plays an important role in the pathogenesis of the metabolic syndrome. However, the syndrome remains a fairly crude phenomenological description of a phenotype, without a clear unified model of pathogenesis.

Publications abound wherein multiple biomarkers are combined to improve risk prediction incrementally. However, until better pathogenesis-based disease models are discovered, we will most likely be without adequate surrogate markers for cardiovascular outcomes in diabetes. How will such disease models be built, and how will next-generation surrogate markers be discovered? Prior to the sequencing of the human genome, disease models were built largely on insightful clinical observations that were interrogated in the laboratory, focusing on relatively small areas of biochemical and molecular focus. Metabolic diseases caused by enzyme deficiencies, such as Gaucher disease and homocystinuria, serve as good examples of this kind of disease modeling. We now have tools to explore the genome (DNA), transcriptome (mRNA), proteome (protein), and metabolome (metabolites) as they relate to clinical phenotypes. A "bottom-up," or inductive, approach is analogous to a "fishing expedition," wherein very large data sets are interrogated for correlation to a clinical phenotype. An example of this strategy is genome-wide association (GWA) studies that catalog single-nucleotide polymorphisms (SNPs) with disease states or outcomes. In this regard, GWA studies have expanded the number of genetic loci associated with T2DM risk to >10, including loci in and around CDKAL1, CDKN2A/B, IGF2BP2, TCF7L2, and HHEX.[23,24] In many cases, the relationship between disease and gene is obscure, indicating either a false-positive association or a mechanism involved in the disease process that is unelucidated. Further insight can be gained by correlating GWA findings with gene expression or proteomic/metabolomic data. For example, Gieger and colleagues[25] showed that a polymorphism in the FADS1 gene is associated with a phenotype (coronary artery disease), as well as a "metabotype," which, in this case, is a signature of serum glycerophospholipid concentrations. The metabolomic association makes sense in this case, because the FADS1 gene encodes fatty acid delta-5 desaturase, an enzyme involved in the metabolism of long-chain polyunsaturated fatty acids. These metabolomic data can thus be very informative *vis a vis* putative genotypic and phenotypic associations.

On the other hand, a "top-down," or deductive, approach builds on knowledge and observations around a biological process, extending current understanding. The deductive approach has yielded a plethora of cancer-associated biomarkers, based on progressive elucidation of the molecular basis of malignancy. This remarkable state of the art began in the early 1900s with the discovery by Peyton Rous that sarcoma in chickens could be transmitted by a cell-free filtrate (i.e., Rous sarcoma virus). The discovery of oncogenes in the 1970s magnified the importance of Rous's earlier work and created a pathway

to understand the molecular basis of cancer. Since 1990, further work has revealed the enormously complex signaling pathways that are initiated by growth factors, vascular endothelial growth factor and epidermal growth factor (for example) interacting with receptor tyrosine kinases, vascular endothelial growth factor receptors and epidermal growth factor receptors, transmitted by protein kinase B, mitogen-activated protein kinase, and others, resulting in dysregulation of normal pathways of cell growth, differentiation, and division.

As information technology expands our ability to generate, store, and interrogate enormous amounts of data and information, inductive approaches to disease modeling and biomarker discovery are becoming more successful. Oncology again provides the best example of this. Commercially available tests such as Oncotype DX (Genomic Health) and Mammaprint (Agendia) correlate gene expression signatures with prognosis and/or likelihood of response to chemotherapy.[26,27]

Efforts to harness massive amounts of data to build better models of disease pathogenesis are underway in government, academia, and the private sector as well. Furthermore, companies such as Entelos, Pharsight, and Optimata are well established in this arena and focus specifically on drug development.

A network approach to disease modeling should elucidate new targets for drug discovery and new biomarkers in turn.[28] For example, using gene expression array technology in various tissues, Keller and coworkers[29] demonstrated the importance of cell cycle regulatory genes in susceptibility to obesity-dependent diabetes in a mouse model. The findings suggest a molecular mechanism to explain the differential ability of islet cells to proliferate in response to obesity in strains of mice that are differentially susceptible to obesity-induced diabetes. These insights have been expanded to human studies in which SNPs were correlated with RNA expression array data from liver and adipose tissue. These data were then integrated into a pathway-based GWA analysis. Type 2 diabetes mellitus was shown to be associated with pathways already known to be associated with the disease, such as peroxisome proliferator-activated receptor signaling, calcium signaling, tumor growth factor beta signaling, cell communication, and pancreatic cancer pathway.[30] However, additional less-characterized candidate pathways, including tight junction, adherens junction, complement and coagulation, and antigen processing and presentation were also implicated.[30] Some of these pathways have been linked to complications of diabetes as well as in the pathogenesis of type 1 diabetes but now serve as candidates for a pathogenic role in T2DM as well.

Finally, imaging data can be integrated into disease models along with molecular and biochemical data. Although few studies have yet achieved this level of data integration, the opportunity is clear. Noninvasive imaging techniques can demonstrate pancreatic beta-cell mass, skeletal muscle lipid levels, and arterial stiffness among other variables.[31–33] Intermediate phenotypes that integrate data from sources as disparate as nucleic acid sequencing and magnetic resonance imaging will further advance our ability to more accurately assess cardiovascular risk in the context of diabetes.

Historically, biomarkers have been discovered by physiologically based associations between some measurable biologic parameter and a disease state or phenotype. A number of counterintuitive biomarker changes in clinical trials demonstrate that disease models are often incomplete or flawed. Improved models of disease pathogenesis will naturally reveal new and improved biomarkers as well as targets for intervention. As molecular and genomic technology advance in step with exponential increases in information technology and analytics, such models are beginning to emerge.

In diabetes, clinical trials have called into question the relationship between the classical biomarker of glycemic control, HbA1c, and cardiovascular outcomes. Methodological advances from other fields, especially oncology, are beginning to pave the way toward improved models of diabetes pathogenesis and biomarker discovery. In this regard, a systems biology approach promises to delineate the complex genetic and physiologic interplay between diabetes, metabolic syndrome, and ischemic cardiovascular disease.

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