Meeting Report

Translational Biomarkers: from Preclinical to Clinical a Report of 2009 AAPS/ACCP Biomarker Workshop

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Abstract. There have been some successes in qualifying biomarkers and applying them to drug development and clinical treatment of various diseases. A recent success is illustrated by a collaborative effort among the US Food and Drug Administration, the European Medicines Agency, and the pharmaceutical industry to provide a set of seven preclinical kidney toxicity biomarkers for drug development. Other successes include, but are not limited to, clinical biomarkers for cancer treatment and clinical management of heart transplant patients. The value of fully qualified surrogate endpoints in facilitating successful drug development is undisputed, especially for diseases in which the traditional clinical outcome can only be assessed in large, multi-year trials. Emerging biomarkers, including chemical genomic or imaging biomarkers, and measurement of circulating tumor cells hold great promise for early diagnosis of disease and as prognostic tests for managing treatment of chronic diseases such as osteoarthritis, Alzheimer disease, cardiovascular disease, and cancer. To advance the success of treating and managing these diseases, efforts are needed to establish the temporal relationship between changes in inflammatory or imaging biomarkers with the progression of the chronic disease, and in the case of cancer, between the extent of circulating cancer cells and tumor progression or remission.

KEY WORDS: biomarkers; diagnostic; diseases; gene expression; imaging.

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INTRODUCTION

In recent years, the success rate of drugs approved for use has not improved compared to that entering clinical development. The costs of clinical development of a drug candidate are substantial, and the R&D expenses spent on those drug candidates that fail late in clinical development are tremendous (1). However, with rapid advances in basic biomedical science, opportunities for finding new therapies are increasing. The effective utilization of biomarker approach during drug development as well as post-approval holds great promise for optimizing the safety and efficacy of pharmacotherapy and individualizing medical treatment to the patient. These biomarker approaches (including genetics, genomics, gene expressions, proteomics, and imaging) will facilitate evaluation of disease progression, drug efficacy, and drug-induced adverse reactions. Drug-related pharmacodynamic and disease-related biomarkers can be used in early drug development to understand and predict patient population characteristics, optimize dosing, and improve the product decision-making process throughout critical phases of development. For the biomarker approach to be effectively translated into the clinical setting, several factors need to be

present; these include the establishment of preclinical bridging biomarkers, validation of the biomarker assay, and clinical qualification of the biomarker.

The US Food and Drug Administration (FDA) has published its Critical Path Initiative and is actively working with experts from academia and industry to facilitate biomarker qualification for drug development and clinical application. To further exchange of information, the FDA and the American Association of Pharmaceutical Scientists jointly developed and presented a workshop entitled “Translating Biomarkers for Accelerating Drug Development: From Preclinical to Clinical”, May 6–7, 2009, in Baltimore, Maryland. The main themes of the workshop were to discuss challenges and successful examples of bridging non-clinical biomarkers to clinical biomarkers covering both efficacy and safety and integrating biomarkers into the development of the drug and the companion diagnostic for personalized medicine. This report describes the proceedings at the meeting and the discussions during the panel sections. Summarized discussions and comments included herein are not to be regarded as the consensus reached at the workshop, but rather as a collection of important issues discussed during the meeting.

BIOMARKER QUALIFICATION FOR DRUG DEVELOPMENT

Biomarkers when qualified are invaluable to the development of medicine for treating and managing various diseases. Biomarkers should have consistent characteristics with an acceptable sensitivity and specificity representing a specific toxicity or therapeutic effect of the drug, a specific physiological response to a treatment, a pathological progression, or a physiological factor. Regulatory agencies have worked with the pharmaceutical industry in a collaborative manner to facilitate biomarker qualification for drug development. Regulatory efforts, success examples, and mechanisms for collaboration among the stakeholders are described as follow.

Food and Drug Administration

The FDA has published guidance documents, established the Voluntary Exploratory Data Submission (VXDS) as well as Biomarker Qualification (BQ) programs (2,3), approved pharmacogenomic tests, and incorporated genomic test information into drug labeling to improve safety and efficacy. The FDA’s Center for Drug Evaluation and Research (CDER) is now implementing a formalized BQ program. The BQ program, together with the VXDS process, will aid developers of biomarkers in identifying and gaining the data necessary to establish a specific, scientifically sound role for the biomarker in therapeutic development (termed a qualified context of use). The BQ program is a framework for CDER to perform a rigorous review of the data to formally qualify a biomarker, thus allowing any therapy developer to use this biomarker in the qualified manner without needing to independently produce and submit the data to justify its use.

Biomarkers could identify different disease subsets in which the same clinical syndrome may have a variety of different pathogenesis. Advanced biomarkers may be one approach to

achieving personalized medicine, and may include, for example, identifying a genetic basis for a treatment response that allows distinction between responders and non-responders or between those at risk for a severe adverse drug reaction and those not at risk. The Office of Translational Science (OTS) supports the FDA’s Critical Path Initiative on biomarker qualification through various programs. Specifically, OTS is building capacity, expanding infrastructure, and leveraging the resources of the Office of Biostatistics and the Office of Clinical Pharmacology to facilitate biomarker qualification. These actions will foster between-office collaboration within CDER.

Under the auspices of Office of Clinical Pharmacology, the VXDS program provides biomarker development and sponsors the opportunity to request a VXDS meeting with the FDA. To date, VXDS meetings have been models of collaborative scientific discussions of the type needed to trigger a biomarker qualification request. The VXDS program is also an important prototype for international engagement and alignment between the FDA and the European Medicines Agency (EMA) for sharing of information. VXDS meetings are not part of a regulatory decision-making process, but are useful in the identification of exploratory biomarker candidates for further development under the BQ program. The FDA’s objectives of establishing the BQ program are (1) to support outside groups who are attempting to establish a biomarker for use across multiple drug development programs, (2) to provide an organized structure for interactions in a consistent and responsive manner while minimizing burdens on product-review divisions, and (3) to establish a process for interactions with biomarker sponsors. Biomarker qualification is a conclusion that within a carefully and specifically stated “context of use,” the biomarker has been demonstrated to reliably support a specified manner of interpretation and application. Qualification can be relied upon in the absence of (1) serious study flaws in collecting the biomarker data, (2) an attempt to apply the biomarker outside the qualified context of use, and (3) new scientific evidence conflicting with prior conclusions. The FDA has recently published a draft guidance document related to qualification of biomarkers, which is entitled “Guidance for Industry, Qualification Process for Drug Development Tools and dated October, 2010 (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM230597.pdf>).” This recent draft guidance provides additional information about biomarker qualification.

European Medicines Agency

European Medicines Agency (EMA), which coordinates the licensing of the majority of new drugs in the European Union, is dedicated to supporting the use of biomarkers in drug development. The most promising domain where biomarkers are expected to support drug development is in the identification of optimized target populations, i.e., a subset of patients with a disease in which drug adverse effects are minimized and/or drug efficacy is maximized. Availability of such biomarkers, which are sometimes called theranostic markers, may not only be useful for drug development but may also support appropriate prescribing, helping to reduce the infamous “efficacy-effectiveness-gap.” Initial experience using biomarkers for the identification of a target population proved to be promising. Thirty-three new oncology products

were approved through the EMA's centralized procedure between January 2000 and December 2008. Of these, nine (27%) had pharmacogenomic biomarker implications in their labels. Lessons learned from qualifying these biomarkers are concerned predominantly with the issue of post hoc analysis. Therefore, the need for appropriate regulatory guidance on qualifying pharmacogenomic biomarkers is recognized.

Since January 2009, the EMA has established a formal procedure for "qualification of novel methodologies for drug development" (4). While the scope of the procedure is quite broad, and may include regulatory guidance for development of, for example, novel statistical methodologies, or instruments for patient-reported outcomes, it is expected that the bulk of consultations under this program will be for biomarker development. Interactions between applicants and the EMA will not be product-specific but will address a clearly defined methodology or biomarker that is expected to lend itself to the development of more than one particular drug product. Applicants may be individual companies, university consortia, public/private partnerships, or others. During the initial stages of methodology development, the EMA will provide scientific advice on future studies required for the qualification of a new biomarker or other methodology. Once studies have been performed and results submitted, the EMA will assess available data and provide qualification advice as to the specific purpose for which the methodology is or is not deemed to have qualified. Qualification advice will involve a public consultation step; the methodology should be open for all drug developers and the scientific community will be given an opportunity to comment on the EMA's assessment of a novel methodology.

In addition to this formal, dedicated process, the European Medicines Agency supports biomarker development by a range of projects under the umbrella of the EU Innovative Medicines Initiative (IMI; 5). The initiative is a unique public-private partnership between the pharmaceutical industry represented by the European Federation of Pharmaceutical Industries and Associations and the European Communities represented by the European Commission. Both partners will provide a total amount of two billion Euros for the initiation and conduct of IMI projects (5). IMI aims to accelerate the availability of novel medicines by addressing current bottlenecks and inefficiencies in the drug development process. The EMA has communicated its support of the IMI's goals and will contribute towards individual projects through direct collaboration or guidance, although it is cognizant of perceived or real conflicts of interests that may arise out of its participation.

Preclinical Kidney Toxicity Biomarkers Qualified by FDA and EMA

A successful example of biomarker development for preclinical drug studies is provided by the pilot FDA/EMA qualification process of a set of nephrotoxicity biomarkers proposed and studied by the Predictive Safety Testing Consortium (PSTC; 6). Twenty-three proposed exploratory biomarkers of kidney toxicity were reviewed, and in the end, seven biomarkers were selected by the FDA and EMA as qualified biomarkers for nonclinical nephrotoxicity (Fig. 1). For the use of these biomarkers in clinical trials, both FDA and EMA concluded that such application needs to be considered on a

Case Study in Nonclinical Biomarker Qualification: Biomarkers of Nephrotoxicity from the PSTC

23 Proposed Exploratory Biomarkers of Kidney Toxicity – 7 Prioritized for FDA/EMA Submission

Fig. 1. Biomarkers of preclinical nephrotoxicity

case-by-case basis (7). It is anticipated that further work will explore the usefulness of these nephrotoxicity biomarkers for clinical drug development. The interactions between regulatory and industry representatives within the PSTC consortium, being the first example of this kind, provided a valuable exchange of learning for all partners.

Qualification of Clinical Nephrotoxicity Biomarkers: an Industry's Proposal

The qualification of the specific set of preclinical kidney toxicity biomarkers as described above is encouraging, and the qualification process and discussion involved will serve as the guide for facilitating the successful development of clinical nephrotoxicity biomarkers. However, the critical task of qualification of clinical nephrotoxicity biomarkers remains to be accomplished. This task needs in-depth investigations of the mechanisms of action or pharmacological pathways of toxicity the biomarkers reflect. It remains to be established that these biomarkers adequately select and characterize the severity of human kidney dysfunction within broad and specific patient populations. This work will require the collection of normative data for the translational application of preclinical biomarkers into a clinical setting.

Qualification of a clinical biomarker requires the understanding of its clinical performance with regard to the level of sensitivity and specificity achieved under a specific context of use. Importantly, clinical factors which might interfere with biomarker interpretation and factors which reveal the mode of action or the mode of nephrotoxicity should be thoroughly understood. For each biomarker, a minimum targeted specificity and sensitivity should be defined. Beyond that, the most prevalent and important clinical factors, that have an impact on the minimum sensitivity or specificity of the clinical biomarker, should be identified. Appropriately, the subpopulation with the most common, accessible, and testable factors should be included in the qualification of biomarkers to define the minimum specificity.

Theoretically, clinical trials to qualify clinical renal injury biomarkers could begin with a drug known to have a specific type of renal toxicity followed by other drugs with more than one mechanism of renal toxicity. However, the drugs studied should only inflict reversible and mild renal injury. The study

population could begin with healthy adults followed by patient with mild renal impairment and then by elderly healthy subjects. The design of these clinical trials would be very challenging and should be subject to rigorous debate before approval to proceed.

Precompetitive Collaboration: a Path to Qualify Clinical Biomarkers

Qualification is the evidentiary process of linking a biomarker with biological processes and clinical endpoints such that it can be used as a surrogate endpoint or adopted for regulatory purposes (8). Development and qualification of some of the most useful biomarkers is generally resource intensive and can take years to achieve the necessary consensus status for regulatory use (9). A challenging task facing biomarker characterization is the necessity to integrate the various approaches taken by the stakeholders, including those from the government, industry, non-profit organizations, providers, payers, patient advocacy groups, and academic institutions. This critical need for partnership drives precompetitive collaboration, including the formation of The Biomarkers Consortium, a public-private platform for precompetitive collaboration specific to biomarker research.

The Biomarkers Consortium recently endorsed adiponectin as a predictor of metabolic responses to peroxisome proliferator-activated receptor (PPAR) agonists in type 2 diabetics (10). Previously, the predictive utility of adiponectin was not well characterized. The Biomarkers Consortium analyzed blinded data on 2,688 type 2 diabetics from multiple randomized clinical trials conducted by four independent pharmaceutical companies. Overall, the combined trial results showed clearly that adiponectin increased after PPAR agonist treatment, which correlated with decreases in glucose, Hemoglobin A1c (HbA1c), hematocrit, and triglycerides and increases in BUN, creatinine, and HDL-C. However, of most interest was the early increase in adiponectin with PPAR agonist treatments that predicted a subsequent decrease in HbA1c. Indeed, a logistic regression analysis of the combined study data demonstrated convincingly that the increase in adiponectin predicted the decrease in HbA1c. These analyses confirmed previous relationships between adiponectin levels and metabolic parameters and supported the potential clinical utility of adiponectin as an early indicator of drug response across the spectrum of glucose tolerance.

This example of precompetitive collaboration successfully facilitated resolution of many important clinical questions that would have otherwise been impossible to resolve using the individual study data sets of each company alone. For clinical biomarkers, regardless of whether they are for demonstration of drug efficacy or safety, precompetitive collaboration could be the pathway forward so that cost, data, and knowledge are shared by all involved in drug development.

BIOMARKER QUALIFICATION FOR DIAGNOSTICS

Clinical biomarkers, once qualified, are useful for facilitating drug development as well as for implementing individualized medicine. Companion diagnostics utilizing clinical biomarkers are applied for the diagnosis of disease and for monitoring disease progression. In a specific disease

population, companion diagnostics can help design a specific treatment protocol tailored to achieve the best clinical outcome. The FDA regulation and industry efforts in this area are discussed below.

FDA Regulation for *In Vitro* Diagnostic Devices

The biomarkers used in *in vitro* diagnostic devices (IVD) typically include genomic, proteomic, and metabolomic biomarkers. *In vitro* diagnostic devices are subject to the adulteration provisions of the FD&C Act under Section 501. The intended use determines the type of regulatory submission (premarket application (PMA) or 510(k) submission). For the cystic fibrosis transmembrane genotyping assay with the indication for carrier screening, it requires a 510(k) submission while the same test used for fetal screening requires a premarket application. For a multiplex instrument system with two devices, for example, if used for detecting *BCR-ABL* (an oncogene fusion protein of BCR and ABL) for chronic myelogenous leukemia diagnosis, it requires a premarket application. However, if the same test is used only for detecting *BCR-ABL* during cancer treatment, then a 510(k) application is required.

IVD classification is determined on the basis of risk. Class III IVD is most complex and involves high risk. For example, cancer diagnosis or screening for safety and effectiveness requires a Class III IVD premarket application. Class II IVD is less complex and involves moderate risk. For example, if for the prognosis and monitoring of patients already diagnosed with cancer, it requires premarket notification (510(k)) under special controls. Class I IVD is common and involves low risk devices. Most Class I IVDs are exempt from premarket submission.

Therapeutic decisions driven by companion diagnostics may enhance better safety and efficacy of drugs. The risks for such companion tests are equivalent to those of the therapeutic products. In the development of a companion diagnostic, biomarkers need to be analytically validated before the diagnostic test is used in the pivotal clinical trial of the drug. There are useful publicly accessible documents and guidance on the FDA web pages for reference with regard to IVD and application of companion diagnostics, as listed below.

- <http://www.fda.gov/cdrh>; <http://www.fda.gov/cdrh/devadvice>
- <http://www.fda.gov/OC/combo>
- <http://www.fda.gov/Cder/genomics/pharmacoconceptfn.pdf>
- Guidance for Industry and FDA Staff Commercially Distributed Analyte Specific Reagents (ASRs): Frequently Asked Questions
 - <http://www.fda.gov/cdrh/oivd/guidance/1590.pdf>
- Draft Guidance for Industry, Clinical Laboratories, and FDA Staff—*In Vitro* Diagnostic Multivariate Index Assays
 - <http://www.fda.gov/cdrh/oivd/guidance/1610.pdf>

Diagnostics of Transplant Rejection: Evolving Progress and Success Achieved

Historically, a biopsy of the kidney, heart, or lung was thought to be the “gold standard” of diagnostics in organ transplantation. In recent years, several biomarkers have

been developed that enhance the understanding of the multidimensional genomic system and provide an improved path forward in complex disease states such as organ transplantation and host rejection. Genomic, proteomic, and metabolomic markers are being identified in peripheral blood and tissue that can monitor changes over time, the influence of multiple drugs, and the influence of concurrent and multiple disease processes (11). For chronic rejection in organ transplantation, establishment of multidimensional parameters will become the new gold standard to delineate the progression of complex chronic rejection and to provide insight into how drug therapy can be developed to treat these disease processes.

An unmet clinical need in the care of heart transplant recipients involves the lack of diagnostic information based on quantitative performance metrics that can help guide clinician decision-making in assessments of acute cellular rejection (ACR). The diagnosis of cardiac allograft ACR previously involved a comprehensive clinical assessment using a variety of methods, with an emphasis on endomyocardial sampling via transjugular venous access and acquisition of biopsy specimens. However, many diagnostic tools for ACR not only lack specificity but are clinically problematic due to sampling errors (wrong tissues) and interpretive variability (not sufficient training of interpreters) associated with examining the biopsies. To tackle these issues, a team of academic transplant cardiologists designed an alternative diagnostic strategy.

The investigators of the Cardiac Allograft Rejection Gene Expression Observational (CARGO) Study (12) utilized a translational medicine strategy to address their hypothesis that a unique profile of genes expressed by peripheral blood mononuclear cells could discriminate between the absence and presence of ACR (13). A research protocol was developed using blood samples and clinical data collected from CARGO Study subjects to perform microarray analyses with the goal of identifying candidate gene biomarkers for ACR using leukocyte gene microarrays. The microarray-identified candidate biomarker pool was supplemented by genes implicated in the cardiac ACR literature, and then subjected to quantitative validation by real-time polymerase chain reaction (RT-PCR). Using linear discriminant analysis, the research informatics team deduced a 20-gene algorithm that distinguishes moderate to severe ACR ($p=0.0018$) in stable heart transplant patients.

The results of the genomic analyses were submitted to the FDA in a 510(k) submission, and resulted in the FDA's classification of the ACR gene algorithm as a Class II device and clearance of the AlloMap® Molecular Expression Test. The test is intended to be part of the standard clinical assessment following transplant surgery and to help identify the subgroup of patients with a low probability of moderate to severe ACR in stable cardiac allograft recipients. Since 2005, the AlloMap test has become a component of patient management protocols at US heart transplant centers, with more than 16,000 tests performed at 90 hospitals and clinics.

A Success Story of Personalized Medicine and Companion Diagnostic

Somatic mutation analysis is a subset of genomic biomarkers that is gaining considerable attention now. This interest will no doubt continue to rise in the near future due to the

advent of next-generation sequencing technologies and their application to analysis of clinical samples. The contribution of somatic mutation to oncogenesis has been studied intensively for decades. Despite this extensive knowledge, data supporting the clinical utility of only a few somatic mutations have been generated to conclusively demonstrate the mutation's ability to predict response to specific therapeutics. Sample access (including quality and quantity), sample heterogeneity (tumor, stromal, and necrotic cell proportions and potential tumor cell heterogeneity), and other technological limitations have no doubt led to this limited success.

The oncogene *KRAS*, which encodes a G protein downstream of the epidermal growth factor receptor (EGFR) pathway, has been well-known for a long time for its potential utility in metastatic colorectal cancer (mCRC) treated with anti-EGFR therapy (14). *KRAS* mutations impact the nature of CRC cells and the aggressiveness of the mCRC disease. Despite its demonstration as an oncogene for 30 years and the apparently obvious role of activating mutations in rendering EGFR-pathway-dependent tumors resistant to anti-EGFR therapy, three issues confounded the potential utility of *KRAS* as a negative predictor for anti-EGFR therapy. First, the efficacy of anti-EGFR therapies was demonstrated in preclinical models utilizing human xenograft models including some carrying *KRAS* activating mutations. Second, the potential prognostic role of *KRAS* in CRC has been debated in the literature for a number of years (thus correlations between *KRAS* and poor outcome may be due to its prognostic value not its negative predictive value). Lastly, early clinical trials of anti-EGFR therapies without control arms (some in combination with chemotherapy) reported some objective responses in patients carrying the *KRAS* mutations (15). Thus, what may have been considered an obvious hypothesis was clouded. However, due to the biological plausibility of *KRAS* and the availability of a sensitive allele-specific polymerase chain reaction-based assay for detection of *KRAS* mutations, the hypothesis was tested on the samples from a completed randomized controlled trial (RCT) in a prospective manner. With the remarkably high tissue ascertainment, *KRAS* status was obtained from 92% of patients participating in the RCT. The progression-free survival rates resulting from panitumumab treatment (208 patients) were significantly higher in wild-type *KRAS* individuals than in mutant carriers ($p<0.0001$). It was clearly demonstrated that *KRAS* is a negative selection biomarker for anti-EGFR therapy (15). It is important to note that this represents an ideal case for a prospective analysis of samples from a completed clinical trial—biological plausibility, a sensitive assay conducted with appropriate validation, high ascertainment (no doubt due to the tumor type, mCRC), a frequency of the biomarker of 43% (thus ensuring balance between the arms despite non-randomization of patient enrollment on the biomarker), and ultimately, an unequivocal interaction between *KRAS* mutation and non-response.

EMERGING BIOMARKERS

As science advances, newer cutting-edge biomarkers are emerging and being developed for drug development, for diagnosis and treatment of the diseases that are still full of clinical challenges, such as breast cancer, osteoarthritis,

Alzheimer disease, and cardiovascular diseases. In the following, the development of emerging biomarkers including gene expression profiling, imaging biomarkers, proteomic biomarkers, and circulating breast cancer cells will be highlighted along with the challenge associated with their qualification.

Chemical Genomic Biomarkers: Bridging Preclinical to Clinical for Drug Development

Gene expression profiles are being used for candidate selection in drug development. The development or clinical manifestation of a disease involves a network of biological factors/pathways, some of which could be downregulated while others upregulated. Gene expression at the transcriptional level could be easily determined using microarray or polymerase chain reaction and is being actively used for understanding the onset and progression of a disease and the pharmacological effects of a new drug. Comparison of *in vivo* gene expression profiles of a specific cell type from an organ or tissue after exposure to a new drug candidate and after exposure to a gold standard is being explored for early candidate selection.

Gene Transcription Profiling as Pharmacodynamic Markers

The corticosteroids have diverse anti-inflammatory and immunosuppressive effects, but also produce numerous adverse effects owing to alteration of genes controlled by the natural glucocorticoid hormone. Studies in the Jusko Laboratory have utilized Affymetrix gene arrays to monitor gene expression under controlled baseline conditions in normal rats as well as after administration of large doses of methylprednisolone (16). These studies have shown that normal rats often exhibit circadian rhythms in gene expression in liver and muscle, but the rhythms may peak at various times of the day. After single-dose exogenous steroid administration, gene expression in liver, muscle, and kidney exhibit response patterns which may rise and return to baseline, but often shows biphasic patterns with an initial decline and a later increase. The latter is a severe complication in use of mRNA as a biomarker. Adding further complexity is that single-dose changes in gene expression are not predictive of responses during chronic infusions (16).

A rat model of rheumatoid arthritis showed considerable promise for use of mRNA as a biomarker for disease progression and efficacy of dexamethasone (17). Genes for the pro-inflammatory cytokines, IL-1 β , TNF- α , and IL-6 in rat paws exhibited a lengthy lag phase and then rose in anticipation of physical changes in paw edema. Dexamethasone caused a sharp drop in cytokine mRNA which was followed by reduction in paw edema. The relative contributions of each cytokine gene could be resolved by pharmacokinetic, pharmacodynamic, and disease modeling of this system.

Use of Gene Expression Profiles in Candidate Selection for Atherosclerosis

A major obstacle for rapid development of new therapies for atherosclerosis is our limited understanding of plaque-

borne biomarkers which are predictive of plaque vulnerability and subsequent clinical events. Currently available molecular markers of plaque composition lack specificity, are highly labile, variable, or not general enough to be used in the clinical setting. As a result, clinical endpoints of cardiovascular disease (CVD) such as stroke, myocardial infarction, and acute coronary syndrome are employed in clinical trials to determine the therapeutic efficacy of a drug. These endpoints require large, long duration trials, thereby increasing both the time and cost of new drug development.

As illustrated in Fig. 2, a key goal was to identify plaque markers of CVD risk and plaque vulnerability, which will facilitate early decision-making for new drug candidates for treatment of atherosclerosis. Gene expression analysis was performed on 925 human peripheral (from lower extremities) and carotid plaque samples. Peripheral plaque was extracted by the FoxHollow SilverHawk catheter and carotid plaque by endarterectomy. The identification of a robust gene expression signature allowed the segregation of plaque samples into inflammatory or non-inflammatory categories. Pathway analyses identified innate immunity, apoptosis, lysosomal dysfunction, and inflammation as major biological processes enriched in this set of genes. The information derived from this inflammatory gene expression profile was used in a clinical trial where patients with bilateral peripheral artery disease were treated for 6 weeks with three commercially available drugs (losartan, pioglitazone, and simvastatin) and plaque samples were taken before and after the treatment. The primary endpoint of the trial, defined as a reduction in CD68 content which is a surrogate for plaque inflammatory status (18), was not observed. This result was most likely due to the complexity inherent to peripheral plaque where samples have different degrees of contamination from media and intima. If this is the case, the use of carotid plaque would circumvent this problem. Interestingly, clear pharmacodynamic markers of drug action could be identified, although a general gene expression signature representative of plaque healing could not be distinguished. In summary, gene expression profiling is an adequate tool to help understand plaque biology. However, integration with data from proteomics and lipidomics studies may be necessary in order to obtain suitable biomarkers of plaque vulnerability.

Imaging and Cellular Biomarkers for Chronic Diseases

Depending on the complexity of the nature of a chronic disease (such as osteoarthritis, Alzheimer disease, and cardiovascular diseases), an accurate diagnosis of the disease stage in individuals often requires concurrent applications of an array of imaging, proteomic, and metabolomic biomarkers. To successfully halt the progression of a chronic disease, a panel of biomarkers along with clinical symptoms is often used to guide the design of a treatment protocol or development of a new drug. Efforts are being made to utilize various technologies to identify a list of essential biomarkers of a diverse nature for this purpose. In treating cancer, a similar rationale also applies since the crucial point is early disease diagnosis and identifying on-target treatment protocols that will be the most beneficial for an individual patient.

Gene expression profiling of human atherosclerotic plaque

Fig. 2. Gene expression profiling of human atherosclerotic plaque

Osteoarthritis: MRI Imaging Biomarkers

Imaging biomarkers play a critical role in both diagnosis and drug development. Current therapies for osteoarthritis (OA), the most common type of arthritis, only provide symptom relief and have no meaningful impact on disease progression. The ultimate goal of OA drug development is to produce a disease-modifying osteoarthritis drug (DMOAD). Such a drug would arrest disease progression, prevent the need for total joint replacement, and improve quality of life. However, this noble effort has been hampered largely by the lack of suitable biomarkers that reliably and precisely measure OA disease progression in a reasonably short period of time.

Imaging modalities are the main technologies for biomarker development in OA. Among them, MRI serves as the most suitable technology capable of identifying a structural endpoint for DMOAD clinical trials (19). Other molecular imaging techniques, such as delayed gadolinium-enhanced magnetic resonance imaging and T1ρ MRI (19), have the potential to overcome the limitations of standard imaging techniques and to reliably identify advancement of structural changes in OA. Results from such enhanced imaging technologies can potentially be used as surrogate markers for disease severity and progression in future clinical trials.

Translational research linking the biology and pathogenesis of OA is needed to advance the discovery, character-

ization, validation, and qualification of biomarkers for DMOAD development. Considering that significant resources are needed to validate such biomarkers, precompetitive collaboration and partnership between public and private sectors are needed. The Osteoarthritis Initiative coordinated through National Institute of Health in the USA is a prime example of this kind of collaboration (20). Most importantly, proactive engagement with regulatory agencies will be critical to qualify a biomarker as a surrogate end point in clinical trials for DMOADs development.

Alzheimer Disease: Cerebrospinal Fluid Molecular Biomarkers and Positron Emission Tomography

Dementia of the Alzheimer's type (DAT) is the cause of approximately 80% of dementia in adults over age 65. At present, a definitive diagnosis of Alzheimer disease (AD) pathology can only be made after death. Diagnostic accuracy during life can be 90% at specialized centers, but it is likely much lower in a community health care setting. Diagnosis is particularly challenging at milder stages of disease, a time when treatments would likely be most effective. AD pathology probably begins years before even mild impairment is detectable. There are no diagnostic tools to detect such presymptomatic individuals or to predict the rate of disease progression in symptomatic individuals. Cerebrospinal fluid (CSF) is made in the brain and can be safely sampled via

lumbar puncture. Recent studies have suggested that levels of disease-relevant CSF biomarkers such as amyloid beta peptide 1–42 (A β 42), tau, and phosphorylated tau (p-tau) may increase diagnostic accuracy (21–24). Imaging studies using amyloid-binding positron emission tomography (PET) ligands may also be able to detect brain amyloid in mildly impaired or presymptomatic individuals (25). Levels of CSF biomarkers correlate with levels of brain amyloid as measured using PET. Levels of these CSF biomarkers and amyloid imaging may predict whether asymptomatic individuals will develop DAT and levels of CSF biomarkers in individuals with very mild dementia may predict rate of subsequent disease progression.

Clinical trials in DAT are particularly challenging because of diagnostic uncertainty and variable rates of disease progression. CSF biomarkers may be useful in the clinic setting as diagnostic and prognostic tools and could be useful as inclusion criteria in clinical trials since they identify individuals who are more likely to progress. CSF and imaging biomarkers may also serve as surrogate endpoints in clinical trials for drug development.

Cardiovascular Disease: from Ultrasound Carotid Artery Intima–Media Thickness to Three-Tesla Magnetic Resonance Imaging

Increased LDL-cholesterol is a classical, well-established risk factor for cardiovascular diseases, myocardial infarction, heart failure, and stroke. Pharmacotherapeutic as well as lifestyle and dietary interventions have been proven effective in reducing LDL-cholesterol levels in at risk patients. However, there has been a lack of easily acceptable endpoints linking the degree of LDL-reduction to the effect on the formation of atheromatous plaques and the underlying cause for cardiovascular events. Thus, studies to show clinical efficacy of novel therapies used morbidity and mortality as endpoints and required large numbers of study subjects (>10,000) and prolonged study periods (>5 years).

More recently, the combined thickness of the intima and media of the carotid artery as assessed by B-mode ultrasound has been used as a noninvasive surrogate biomarker to screen for and monitor atherosclerosis. The predicative value of carotid artery intima–media thickness (CIMT) for cardiovascular disease has, for example, been shown in the Cardiovascular Health Study (26). The application of CIMT as an efficacy marker for lipid lowering drugs is not without challenges. Besides others, the ASAP trial (effects of Atorvastatin *versus* Simvastatin on Atherosclerosis Progression) comparing simvastatin to atorvastatin (27) and the ENHANCE trial (Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression) comparing the combination of simvastatin and ezetimibe to simvastatin in familial hypercholesterolemia used CIMT as primary outcome measure (28). Following a 2-year treatment of simvastatin, the ASAP trial showed increased intima–media thickness while the ENHANCE trial showed reduced carotid artery intima–media thickness. Comparing the divergent results of both trials suggests that patient population have to be carefully selected based on their pretreatment level and disease progression status. Pretreatment with standard-of-care statins results in plaque delipidation in aggressively pretreated individuals, and

substantially reduces disease progression as quantified by CIMT increase. Thus, therapeutic effects become increasingly difficult to differentiate between treatment groups. Ideally, the control group treated with the reference intervention should still have disease progression and the population should have a lipid rich intima at study initiation, so that treatment may result in quantifiable CIMT reduction.

One of the limitations of the CIMT assessment by ultrasound is its lack of sensitivity as measurement results may be dependent on the angle at which the carotid artery is scanned. The 3-Tesla magnetic resonance imaging (3T MRI) is an emerging alternative to CIMT as standard procedure to assess cardiovascular disease (29). Mean wall thickness as assessed by 3T MRI was shown to be highly correlated with CIMT assessed by B-mode ultrasound. The 3T MRI-based approach, however, has substantially better reproducibility, thereby leading to reduced sample size requirements for studies designed to detect treatment effects for novel medications in cardiovascular disease.

Breast Cancers: Circulating Tumor Cells

Circulating tumor cells (CTCs) represent an important biologic link in the spread of breast cancer (BC) from primary to metastatic disease. Multiple methods are used to detect CTCs. Density gradient centrifugation, immunomagnetic capture, and size filtration methods have been used to isolate cells. Microscopy or RT-PCR has then been commonly used to detect/quantify the isolated cells. Tumor cells are also detected in the bone marrow, referred to as disseminated tumor cells (DTCs). CTCs and DTCs are being investigated in both early stage and metastatic breast cancer (30,31). The early breast cancer setting has primarily utilized DTCs or RT-PCR-detected CTCs. Both methodologies identify patient subgroups that are at high or low risk of recurrence.

In metastatic disease, CTCs have been studied primarily using immunomagnetic isolation followed by immunofluorescent microscopy. CTCs in the metastatic setting are both prognostic and predictive. When collected after one cycle of therapy, CTCs appear to identify patients on ineffective therapy. Patients with elevated CTCs after one cycle have median progression-free survival and overall survival of approximately 2.1 and 8.2 months, respectively, when measured from baseline. Patients who convert from high to low CTCs after one cycle of chemotherapy had a much better prognosis, with median progression-free survival and overall survival of 7.6 and 14.6 months, respectively (31). This observation has led to the SWOG S0500 trial (32), in which patients with elevated CTCs 3 weeks after starting chemotherapy are randomized to continue current therapy *versus* switching immediately to a new chemotherapy.

CTC research now focuses on the detection of biologic markers such as estrogen receptors, progesterone receptor, apoptosis, B cell lymphoma 2, amplification of human epidermal growth factor receptor 2, mutations in epidermal growth factor receptor, telomerase, and others. Analysis of these markers may provide invaluable insight into the mechanisms of metastasis, identify novel therapeutic targets, and provide the ability to monitor targeted therapies.

SUMMARY OF KEY DISCUSSIONS AT THE WORKSHOP

1. The application of surrogate endpoints in place of clinical efficacy endpoints to ensure a successful demonstration of the efficacy of a drug remains very challenging, especially for treating chronic diseases. Probably, the most controversial application of biomarkers for drug development is in the area of surrogate endpoints. Thorough understandings of the progression of the target disease and of the biological pathways targeted by the drug action may reduce the false paths in efforts to qualify surrogate endpoints. The potential value of fully qualified surrogate endpoints remains undisputed, especially for a range of diseases where the clinical outcome can only be assessed in large, multi-year trials. However, recent history has not seen great advances in achieving this goal. Some surrogates under development (e.g., imaging in multiple sclerosis), but also well-established surrogates (e.g., blood glucose control), have been called into question. The issues include the concern that off-target effects may not be captured by surrogates, that efficacy surrogates do not indicate safety, and that fully qualifying biomarkers as surrogates may be as difficult and time-consuming as conducting clinical endpoint trials. While it is difficult to speculate on future evolution in this area, qualifying surrogate endpoints is not an easy shortcut for drug development.
2. Companion diagnostics is an essential part of personalized medicine; efforts on qualifying biomarkers should aim at both drug development and clinical diagnostics in order to achieve the most clinically rewarding outcomes.
3. In addition to being impacted by circadian rhythms, gene expression can be subject to other environmental influences. Cross-laboratory consistency in data quality is another challenging issue. Due to the complexity and variability of gene expression profiles, combination with other biomarkers is necessary to successfully utilize microarray data for drug development. Establishment of a set of criteria for choosing the gold standard when utilizing gene expression technologies is as critical as defining the experimental parameters and conditions in which gene expression studies are to be conducted. At the early development stage such decision can be easily made within each company, but the ultimate decision of using these results to move a drug into late stage clinical trials would need sound, convincing scientific evidences.
4. Application of imaging and circulating cancer cell biomarkers to both drug development and companion diagnostics is promising, yet the broader uses of these biomarker technologies face the obstacle of how to effectively integrate them into the clinical management of chronic diseases. The reasons include i) the technologies are relatively new and continuously evolving, ii) the association of these biomarkers with the clinical efficacy endpoints of treating complex chronic diseases remains to be established, and iii) these biomarkers need support of other biomarkers such as proteomic biomarkers while the latter also require rigorous qualification. The temporal

associations between circulating inflammatory biomarkers and image biomarkers in relation to the clinical progression chronic disease remain to be established. Such associations are vital to establish the usefulness of these biomarkers as surrogate endpoints providing proof of the clinical efficacy of a drug candidate.

5. It is recognized that clear communication and transparency between all the parties involved and across disciplines for precompetitive collaboration hold the key to achieving an agreed-upon common goal. Though there are still many challenges ahead, the willingness to collaborate among all the stakeholders is tremendous.

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