Medical applications of microarray technologies: a regulatory science perspective

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The potential medical applications of microarrays have generated much excitement, and some skepticism, within the biomedical community. Some researchers have suggested that within the decade microarrays will be routinely used in the selection, assessment, and quality control of the best drugs for pharmaceutical development, as well as for disease diagnosis and for monitoring desired and adverse outcomes of therapeutic interventions. Realizing this potential will be a challenge for the whole scientific community, as breakthroughs that show great promise at the bench often fail to meet the requirements of clinicians and regulatory scientists. The development of a cooperative framework among regulators, product sponsors, and technology experts will be essential for realizing the revolutionary promise that microarrays hold for drug development, regulatory science, medical practice and public health.

The hybridization of analytes in a single sample to thousands of different specified targets simultaneously on a microarray has become central to genomics research and is now being applied in the field of proteomics. Arrays of oligonucleotide or DNA sequences are being used for genome-wide genotyping[1–9] and expression profiling[10–32], and several potential clinical applications have begun to emerge as our understanding of these techniques and the data they generate improves. Protein microarrays comprised of antibodies, aptamers, whole cell or microdissected cellular lysates, recombinant proteins, small-molecule drugs, phage and antibody-like molecules are being actively explored and used for multiplexed proteomic based endpoints[33–40]. Regardless of the application, the resulting information can comprise thousands of individual measurements and provides an intricate and complex snapshot of biological properties of the cell, tissue or organ with profound significance.

The success of fully exploiting these powerful approaches depends on several criteria: the accurate selection, amplification and location of probe molecules; accurate reference sequence information; identification of unique oligonucleotides; accurate distinction among multiple products of a single gene; accurate reconstruction of expressed sample nucleotide sequences; precision image scanning; and reproducible and accurate transformation of image files to numerical data. For DNA and protein microarrays to be reliable tools, they must possess probe sequences that hybridize with high sensitivity and specificity, thereby allowing precise detection of their intended targets. Results must be highly reproducible, and quality control and quality assurance systems must be established. Determining the appropriate level of analytical and biological validation needed for each medical application of microarrays and their supporting computer based bioinformatics systems[41] raises new challenges for scientists in industry, academia and regulatory agencies.

Drug development and medical practice are likely to be improved by the identification of genes and proteins that are linked to adverse events, differential responses at desired drug targets, disease and alterations in normal drug metabolism. The Food and Drug Administration (FDA), in anticipation of the expansion of microarray-based technologies and the applications of subsequent developments, uses as a guiding principle an analysis of the benefits versus the risks for each new product.

Although the benefits of genomics and proteomics to public health are potentially enormous, challenges must be met to insure a seamless incorporation of these technologies into product development, evaluation and regulation, and into medical practice. Mutual scientific understanding of strengths and limitations of these technologies must be shared throughout the community in an open and transparent way. Regulators, spon-

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sors, and academic researchers have a common responsibility to incorporate the best scientific practices into product development and clinical practice when those practices are sufficiently mature and well understood. To achieve this, early flexibility is important and must be balanced against a consensus that is based on sound science.

Applications of content-driven genomics and proteomics

Solutions to the regulatory challenges will not be the same for all applications of genomic and proteomic microarrays but, instead, are likely to be highly dependent on context. Two important questions will probably affect how microarray data are scrutinized. First, during which stage of drug development are the data derived? Second, how will the data be used? Thus, the level of scientific rigor for microarray performance is likely to differ depending on whether the microarray is being used for early drug discovery and hypothesis generation or as a clinical device to make diagnostic, therapeutic or prognostic decisions for patients. We foresee six main applications of microarrays at different stages of drug development (these are discussed below). But even within these application areas, individual cases are likely to define their own specific scientific issues and the degree of regulatory oversight.

Assessing RNA and protein alterations in early drug screening. Greater mechanistic understanding, broader identification of target tissues, more global and earlier assessments of likely long-term exposure consequences, and improved interspecies biomarker linkages can facilitate compound selection and reduce ambiguities during drug development that occur, for example, when toxicities are seen in some but not all test species and when the relevance to humans is unclear.

Assessing RNA and protein alterations in nonclinical toxicology studies. Drug development protocols for new products are beginning to include genomic and proteomic microarray data obtained during preclinical stages of investigation. Currently, extrapolating this information to humans is not straightforward. As the science evolves, however, such data may provide greater insight into and better prediction of the performance characteristics of the product as it moves into clinical phases of development.

Assessing quality control of cell substrates for manufacturing biologicals. Genomic and proteomic based methods could become components of quality control tests to improve verification of the identity, purity, safety and potency of products such as vaccines, blood derivatives, complex protein mixtures, cells and biological therapeutics.

Assessing RNA and protein alterations in clinical samples as diagnostic biomarkers. Measurements of genomic and proteomic alterations may be used to aid in risk assessment of patient subpopulations, to establish more specific diagnoses, to select optimal therapies and to monitor patients' response to therapies, and for a broad variety of diseases, most notably cancer[11–32,42,43]. Clinical trials of new drugs and biologics present unique opportunities for concomitant studies of diagnostics, including ones developed using microarray technologies, in a manner that meets both scientific and regulatory needs. Failure to address diagnostic issues when designing studies that promise new therapies can delay the scientific development and regulatory approval of the new diagnostic, make full characterization of the new diagnostic impossible owing to a loss of controlled patient samples and compromise evaluation of the therapy itself.

Until recently, submissions for the premarketing review of diagnostic devices involved the evaluation of one analyte, chemical, microorganism, protein, and so on, which resulted in one data point per sample. This number has increased up to only several dozen analytes for more complex hematological or chemical profiles. The FDA anticipates that in the future, data for both the development and evaluation of the effects of drugs and biologics and for certain diagnoses will be derived from patterns of hundreds to tens of thousands of signals measured concomitantly with new mulitparametric technologies. FDA strongly encourages collaboration between the manufacturers of *in vitro* diagnostic devices and drug or biologic manufacturers when appropriate, and recommends interaction with the relevant FDA regulatory centers early in the product development process. Existing frameworks, such as early stage pre-Investigational Device Exemption meetings or device protocol reviews, pre-Investigational New Drug Application meetings and pre-Biologic Licensing Application meetings can offer opportunities for sponsors to receive nonbinding feedback regarding performance expectations of new technological applications and can also be a means of establishing ongoing dialogue with the FDA.

Assessing critical regions of a pathogen's nucleic acid sequence in clinical studies. The FDA anticipates that microarray data will be used to diagnose infectious diseases and, for example, to direct antiviral therapeutic options based on nucleic acid sequence information.

Assessing critical regions of inherited somatic cell DNA sequence in clinical studies and patient-tailored therapy. In the diagnostic assessment of inherited somatic cell DNA sequences, rigorous analysis of the clinical impact will depend not only on the ability of a new diagnostic to reliably detect importance sequences but also on the manner in which these sequences are expressed biologically. Penetrance and expression, interactions with other sequences and/or environmental or biological factors, and the potential for biological and analytical interference and variability must be taken into account as interpretative guidelines are framed for gene discoveries. Ambiguities may be anticipated in defining both haplotypes and the strength of phenotypic associations. Criteria for selecting clinical trials participants in many instances could come to rely more heavily on pharmacogenetic testing. Analyses of trial outcomes could take into account both the level of benefit afforded by the patient definition gained by these technologies and the impact of these technologies on product approval and labeling, and eventually on clinical practice. Genome-based microarray devices could become valuable tools for identifying patients at risk of developing life-threatening reactions during clinical trials[44].

Challenges that microarrays present to regulatory scientists

The primary impact of gene and protein microarrays on medical practice will be the ability of the investigator to collect, from a single sample, multiparametric data sets on a far greater scale than previously possible. The volume and breadth of the data alone mandate the application of sophisticated creative computer algorithms[41,42,45,46] and invite numerous views on the interpretations of biological meaning. In addition, even a very small error rate applied across such large data sets can result in a significant number of 'false positive' signals.

Regulatory scientists could facilitate implementation of technologies that, like microarrays, provide volumes of useful and pivotal multi-analyte information. But on the other hand, misleading information could introduce chaos into a time-tested system that assures consumers that pharmaceuticals, biologics and devices provided by trained health professionals will improve their health.

Reasonable concerns exist about the use of data derived from global array technologies for medical applications. While microarray expression data are improving, there is currently no

Box 1. Challenges for integrating microarrays into drug development and medical practice

Scientific community
- Demonstrating the strength of the linkage of genomic and proteomic measurements to associated biological outcomes
- Demonstrating sufficient sensitivity, specificity, reproducibility, robustness, reliability, accuracy, precision and clinical relevance of the chosen microarray platform application

FDA
- Developing early working relationships with stakeholders to provide reasonable and appropriate context-specific expectations
- Developing for each application objective, fair, consistent and critical risk-benefit analyses of the value and impact of new multiparametric proteomic and genomic technologies on both product development and patient care

convincing evidence to support a high level of intralaboratory reproducibility, reliability, precision and accuracy of data derived from global gene expression technologies applied across platforms to identical samples[47–50]. The validity of conclusions based on systematic computational methods applied to large data sets to extract medically useful information has been criticized[51]. However, data demonstrate that gene expression profiling can be applied in very convincing and reproducible ways to investigate drug actions nonclinically[52–56] and clinically, to stratify and predict patient response[17,21,25–30] and to further understand and diagnose disease[16,18–20,22–24,31,32,57].

The most critical issues that need to be resolved pertain to the reliability of the information and to the development of appropriate controls and references. Standards related to reliability of new and evolving technologies are achievable when experts in government, industry and academia share their expertise toward developing a consensus. Three general and overlapping issues will need to be resolved carefully for each application; these are discussed below and summarized in Box 1.

Hypothesis generating data. Our understanding of gene function and gene product interactions is evolving rapidly. Genome-wide data collection by scientists with a specific hypothesis in mind can be re-analyzed and re-interpreted by others to make alternative assessments as the biological understanding of gene products evolves. Our ability to measure end points has outpaced the ability of science to explain all of them convincingly. The huge scope that microarrays provide requires data-reduction applications that fine-tune and filter the raw data and then manage, analyze, visualize, comprehend and communicate the data output.

A consequence of simultaneously investigating more complex endpoints is the fear that results that are not be easily explained or reliably reproduced could raise concerns with regulatory authorities. A study showed weak agreement between gene expression data from microarrays and data obtained with alternative analytical approach[58]. On the other hand, a more recent study reported a high correlation ($R = 0.76$) in the expression ratios of 54 genes in two samples measured by both oligonucleotide microarray and qRT-PCR[59]. However, careful examination of the data found evidence of potential false positive signals, whereby the expression of approximately 7% of genes appeared significantly altered in opposite directions using the two methods. In another study, microarray-based expression ratios of seven genes measured in two sets of samples were confirmed using qRT-PCR with very good concordance and only general evidence of some discordant data compression in the microarray data[56]. The assessment of ten genes monitored across eight sets of samples supported the accuracy of microarray data, again with evidence of microarray data compression and roughly 5% (4 of 80) of the measurements appearing to be discordant only at low ratio changes[54]. Potential reasons for these discrepancies include variable cross hybridizations of probes with sample transcripts of high sequence homology, inaccurate sequence database annotations, or the presence of splice variants. Indeed, roughly 10% of clones in a commercial set used for an array were found to be sequence annotated inaccurately[54], and approximately 50% of expressed eukaryotic genes are estimated to be expressed as splice variants[60].

There are also concerns about data being overinterpreted. For example, a recent study using microarrays has confirmed by independent methodologies that peroxisome proliferators and phenobarbital are associated with sustained increases in cyclin B and other genes associated with cell-cycle regulation and DNA synthesis[54]. The authors reasonably suggest that these changes may contribute to sustained hepatocellular proliferation and the later appearance of rat liver tumors, which are known to be dose-dependently associated with these agents. But does this mean that all such changes in cell cycle and DNA synthesis transcript profiles can predispose to tumor propensity and thereby define a 'signature' safety concern? Fundamental knowledge, reliable data, accumulated experience and good judgment are essential to avoid raising false concerns, to evaluate links between gene expression alterations and biological outcome, and to recognize legitimate toxicological responses.

On the other hand, the availability of formats that identify changes without knowing which are cause and which are effect challenges scientists to consider the acceptance of experiential correlations under certain conditions in which highly beneficial effects may be realized[43], even though the logical connections may not be apparent immediately. Similarly, associations of identified single-nucleotide polymorphisms (SNPs) in linkage disequilibrium with functionally altered phenotypic outcomes[61] may show very strong statistical associations but may not represent the exact genetic cause[62].

There are two broad types of linkages between SNPs and clinical phenotypes. The first and more persuasive type requires a well-understood, genome-based pathophysiological mechanism to predict differences in clinical phenotypes. In the second type, associations between SNPs and clinical phenotypes are observed without a clear pathophysiological mechanism. This type of linkage is less desirable but may be convincing with a more substantial dataset. In either case, the finding of linkage needs to be replicated, although the extent of the replication required may depend on what is known about the underlying mechanism for the association. One limitation of the second type of linkage is that extrapolating the findings beyond the population that was studied becomes more difficult. Whether SNPs become surrogate markers for disease will depend on several factors, but principally on the degree to which changes in SNP markers can predict changes in clinical outcome. To help make these value judgments wisely and confidently, reliable experimental data, reliable data reduction algorithms, fully integrated traditional data sets and publicly available and scientifically verified referenced data sets may be needed. Easily queried strong experiential reference databases may be crucial for achieving specific goals.

Variable imprecision. Individual measurements from a single microarray platform do not share the same precision, sensitivity or specificity. For example, even for a microarray with 99% accuracy ($P < 0.01$), readouts of 10,000 data points would still yield 100 false positive signals based solely on random chance. For DNA microarrays that detect sequence variations, algorithms are being designed[9] to identify regions of genes that allow greater than 99.9% accuracy, to exceed the natural rate of variation of approximately 8 differences in sequence for every 10,000 bases among individuals. For expression arrays, the annotations across

different platforms are not represented by exactly the same gene sequence regions. Competing sequence targets vary from tissue to tissue and from sample to sample, thereby adding to variability in the hybridization-based measurements for any given probe. Although numerous replicate microarray measurements help reduce imprecision[47,63], the costs of routine extensive replicate experimentation is high and may not always be necessary to answer a specific question.

Commercial expression microarray providers are taking measures to optimize data quality. For example, to assess sample quality and reduce variation, multiple replicates of the same sequence are included on a single array using either the same or distinct probe sequences for the same gene; to assess background hybridization and lower limits of sensitivity, negative control probe sequences of bacterial genes are included; to assess assay performance and signal linearity, positive control bacterial probes and sample spikes are included; to minimize and assess cross hybridizations, probe sequence selection is optimized and mismatch probes are included; and to enhance interlaboratory reproducibility, standard procedures are developed and updated.

Bioinformatics approaches to evaluating and quantifying gene and protein expression patterns continue to be developed so that these patterns may themselves be used as a diagnostic endpoint in the future. At present, it is not possible to provide detailed mechanistic explanations for all the observed patterns. Although this limitation may not prevent productive use of the information in some contexts, it may be more difficult to judge the utility of correlative complex patterns without knowing the underlying physiological mechanisms. This may be one of the greatest challenges this field currently faces. Thus, issues such as complex pattern reproducibility, quality control and quality assurance, in-process testing and the development of appropriate standard operating procedures for performing, evaluating, and interpreting gene and protein arrays are of great interest. Scientific consensus and standards are needed to develop, to evaluate and to accept new statistical models for establishing the significance of linking gene and protein pattern analyses to more conventional diagnostic end points or outcomes.

Platform and data maturity. Microarray technologies are in a constant state of evolution, and new developments appear at a regular pace. Evolution is rapid in the analytical integrity of the technologies, in bioinformatics support and in data analysis programs, and yet a lack of universal standardization for both analytical methods and data format and content remains. Protocols are modified regularly, and programs are updated. Numerous platforms are available with probes designed from different gene sequences for targets with the same names. The many alternatives include, for example, cDNA microarrays versus high density oligonucleotide microarrays, spotting versus *in situ* synthesis, other methods for immobilization, single-dye versus dual-dye hybridizations, cyanine dye-labeled versus biotin-labeled nucleotides, several image capture and image analyses options, single versus 2-step dye labeling, numerous methods for normalization, and several methods to amplify small amounts of RNA. Similarly, in the field of proteomics, a plethora of different formats are being considered. Protein arrays consisting of several antibodies or bait molecules recognizing the same protein may be needed to ensure accuracy. Reproducibility, specificity and sensitivity of the capture agents, the huge dynamic range of the proteome, and the lack of a direct amplification system such as PCR all have important effects on the use of protein microarrays as medical devices.

A continually evolving technology presents difficulties for standardization and consensus development. Some of the obvious gaps include a lack of standardization for gene annotations and bioinformatics software. In addition, there are no 'gold standards' such as reference RNA, genes, proteins, body fluids or reference algorithms. Such standards are essential for scientific assessments of data generated in different laboratories and on different platforms.

Each expression profile measured by microarray hybridization represents a single measurement that is of greater value when compared against all other results obtained with relevant objects. Data from individual expression profiles are stored in databases that represent extremely valuable resources. Issues of database design and maintenance are among the central technical issues that need to be resolved before microarrays can realize their full potential for medical applications (see also review by C. Stoeckert, pages 469–473, this issue)[64]. Although large data sets exist in proprietary databases, additional databases have been created and are growing in the public domain at many different universities and government institutions. Such reference databases are essential to judge technology maturity, to evaluate individual experimental integrity and to interpret the biological meaning of experimental results. Issues that will need to be resolved include free access and intellectual property domains, data vetting and curation oversight and management.

Moving forward, looking ahead

Regulatory agencies normally publish guidelines to clarify uncertainties in interpreting regulations and to clarify regulatory expectations in areas critical to drug development. These guidelines should be based on sound science, but they should be fairly general and not too proscriptive, so that new technology can be developed over time. Before such guidelines can be deleveloped for microarray technologies, scientific agreement on outlining a set of best practices for industry needs to be established. As microarray technology is evolving rapidly, and end users are only beginning to learn how to interpret changes in largely unfamiliar study endpoints, establishing a rigid set of proscriptive guidelines may prove to be detrimental because it may limit exploratory research and the advancement of the science. One might argue that guidelines will be more helpful once we have a better understanding of study design, an accurate picture of the limitations of the technology, and improved understanding of data interpretation. On the other hand, it is reasonable to want to establish minimal performance characteristics to demonstrate data integrity and to guide the content and format of data submissions.

Nevertheless, there are many questions that need data to be answered. For example, should every data point be generated in sufficient replicate to represent a reliable measure of precision of that analyte? Should the linear dynamic range for each analyte on a platform be calibrated by manufacturers under diverse conditions of use? Must absolute accuracy for every analyte be defined to be useful? It may be that different degrees of validation, or different assurances of data validity, will be expected, depending on the stage of product development, the question being asked and the role that the microarray data is expected to have in product performance evaluation.

If an array consists of a multiplex of ten outcomes, each component may contribute a relatively large portion to the overall pattern. As the multiplex increases further to include hundreds or tens of thousands outcomes, then the relative contribution of each point diminishes rapidly. When assessing alterations in thousands of RNA molecules at one time, it may be unreasonable to expect all probes and targets to hybridize optimally and for no cross-hybridization to occur. Perhaps then, it is more reasonable to accept that the measure of each analyte may be an approximation, that some measurements will be more accurate than others, and that these differences may vary from experiment to experi-

ment and from platform to platform. But the biological interrelated nature of the alterations is likely to uncover defining and possibly unexpected patterns that may identify a smaller subset of defining analytes that need to be monitored[20,55]. Again, it may be that different levels of independent assurances of the accuracy of measurements of specific analytical endpoints will be expected, depending on the role that the data will have in nonclinical and clinical studies.

A consideration, therefore, is that genome- and proteome-scale microarrays should be used primarily for candidate selection, hypothesis generation, mechanistic investigations and discovering potential biomarker linkages. Under such circumstances, it is not clear whether or when microarray data on new drugs and biologics, generated outside the core safety and efficacy data set, would need to be submitted to regulatory agencies. If it is likely that such data would be viewed as a valuable component of the pharmacology database of the new drug or biologic, then they should be submitted. If multiparametric data are used as a clinical diagnostic tool, then they will have to be rigorously evaluated by the FDA. In general, it is most likely that genomic and proteomic data, if considered useful for explaining the mechanism of drug or biologic action, will also be helpful in enhancing our understanding of the effects reported in animal studies.

A cooperative two-day workshop was held in May 2002 on Pharmacogenetics and Pharmacogenomics in Drug Development and Regulatory Decision-Making (see http://www.fda.gov/cder/calendar/meeting/phrma52002/workbook.pdf) under the co-sponsorship of the FDA, the Pharmacogenomics Working Group (comprising major companies engaged in pharmacogenomics research) and the DruSafe Group of the Pharmaceutical Research and Manufacturers of America. The workshop considered processes by which the FDA and industry can work together to develop mechanisms for systematically sharing and learning from exploratory microarray data from products under development. In addition, through efforts coordinated under the International Life Sciences Institute (http://www.ilsi.org/file/genomics.pdf) and the Human Proteome Organization (http://www.hupo.org), FDA, industry and academic researchers have been cooperating to develop strategies and processes for microarray applications and, in some cases, have been generating, sharing, analyzing and debating interpretations of collaborative experimental data across different platforms.

Individual sponsors are likely to migrate toward a single favored microarray platform to guide their product development decisions and to develop their own internal reference databases. Some will choose a commercially available array, whereas others will develop their own proprietary platforms. Regulatory authorities will learn from data sets derived from different platforms from laboratories presenting data that are associated with similarly labeled gene identities, but may not be measuring the same marker response. The degree of quality control, good manufacturing practices and validation that microarray manufacturers apply to their products may not be equally rigorous across manufacturing platforms. It will be important for microarray providers to share detailed information on manufacturing controls, post-manufacturing lot-to-lot quality control and functional performance/pass-fail measures. Information is lacking on how microarray users establish standard procedures to consistently assure sample quality and calibrate scanning instrumentation to assure integrity of their data sets.

Numerous statistical, image analysis, pattern-recognition and data-reduction clustering algorithms are applied to microarray data. For screening compounds and determining the effects of a drug on a target tissue, applying these algorithms will help to provide 'big picture' categorizations based on similarities to different classes of drugs, but it may also highlight details that could distinguish among individual agents within a class. The biological interpretation(s), regulatory implications and potential legal ramifications of such evaluations of product performance using global gene expression data are not fully evolved, but will probably be clarified as technology improves and our scientific understanding advances.

Toxicogenomic and toxicoproteomic microarray databases are indispensable resources for comparing and contrasting new compounds with paradigm compounds that work through established mechanisms. Databases may be useful for the FDA to place individual microarray results into perspective, although it is not clear how this should be fairly and transparently accomplished and shared in a way that protects the proprietary interests of sponsors. As databases improve and scientific knowledge expands, it is possible that data generated today may become more informative and, thus, more relevant to safety and/or efficacy assessments. At present, the scientific community lacks consensus on the standardized set of information required to fully annotate data generated from microarray experiments. Efforts that are underway[65] to establish worldwide scientific consensus on the minimal information descriptors for such data may provide a universal solution to guide the uniform content of such databases.

Over time, gene expression data derived from standard animal toxicology and clinical efficacy studies may provide those in drug development with strategies to minimize the number of variables in study design, reduce the length of long-term toxicology studies, or limit the numbers of species for long-term toxicology studies. Use of animal toxicogenomic data and early clinical pharmacogenomic and pharmacoproteomic data could help to define both essential sets of biomarkers and efficient strategies for subsequent clinical studies. So far, the FDA and sponsors have had few opportunities to discuss data submissions for products using microarray technology. The agency recognizes the need to develop and learn from a broader experiential and interactive knowledge base derived from the review of submitted data sets.

Encouraging sponsors to explore and to submit microarray data would accelerate and foster dialogue on the meaning of such data. Ultimately, the FDA will best serve the public if it poses the critically important questions to microarray manufacturers and receives the same informative responses that all end users will require. To ensure that the agency does not hinder clinical transfer of this important technology, we are committed to evaluating new diagnostic applications using least-burdensome thresholds, as outlined in the FDA Modernization Act of 1997. In addition, when data sets are small or uncertain, the FDA is committed to transparency in communicating this information to potential users, whether in the feasibility or in the developmental stages of a diagnostic. The agency looks forward to participating in the evolution of medical applications from the new fields of science ushered in by genomic and proteomic microarrays, and by other multiparametric technology platforms[9,12,14,40].

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