pathways, they will need to be translated into more-efficient HTS assays.

'Hits' discovered in such *in vitro* assays will then face the challenge of demonstrating efficacy in disease models *in vivo*. The dose-limiting toxicities that such drugs might incur remain unclear, given that cytoskeleton-dependent membrane trafficking pathways are essential in all cells. Nonetheless, success in finding such a molecule would result in the discovery of a novel class of drugs, exploiting cytoskeleton-dependent membrane trafficking pathways to cure disease.

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From PGx to molecular diagnostics and personalized medicine

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The organization of two related IBC conferences entitled *Molecular Diagnostics* and *Personalized Medicine*, held with concurrent sessions on 28–30 May 2003 in Boston, USA, symbolizes how compelling applications of pharmacogenomics/pharmacogenetics (PGx) in drug development and the practice of medicine are leading the renaissance of molecular diagnostics and the emergence of new regulatory policies.

The conferences brought together key speakers from the major pharmaceutical and biotech companies with PGx strategies incorporated in their drug development programs using enabling technologies for high throughput profiling of genotypes or expression of genes and proteins. The inclusion of topics on regulatory perspectives and reimbursement issues revealed major criteria that would be key to successful commercialization of PGx products in the future. Highlights from select presentations are captured here.

From PGx to better drugs and healthcare

In the new era of personalized medicine, the common goal iterated throughout the conferences – 'right drugs for the right patients' – is the primary driver for PGx applications. Yet molecular diagnostics constituted only 3% of the total *in vitro* diagnostics tests in 2002 and only 1500 genes and 5000 proteins were candidate markers. Failure to predict drug toxicity resulted in 100,000 deaths and 20–40% patients received the wrong drug [1]. With the encouragement from regulatory agencies, the pharmaceutical industry is now embracing PGx as the key to personalized medicine [2]. The goals are to identify the best drug targets for the individual disease cases, reduce toxicity and improve efficacy, determine the optimal drug dosage, timing and route of administration, and eliminate in early phase the development of drugs that will fail in the clinic. The expected results include smaller and less expensive clinical Phase III trials, safer drugs and more effective healthcare. The resurrection of failed drugs for use in a highly stratified patient population will also become possible.

Development of PGx products

There are two major areas where speakers applied PGx to drug discovery and development. One relates to the genetics of the host and the other to the pathology of the disease. Host genetics has significant impact on pharmacokinetics and pharmacodynamics. The static nature of genotypes makes genetics testing relatively easy for clinical use. However, the combined effects of host genetics and various environmental factors add

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dynamic complexity to the etiology of disease. Therefore, in addition to genotyping, many diagnostics and pharmaceutical companies are using genome-wide gene expression profiling of diseased tissues towards deciphering mechanistic pathways and developing differential theranostics for specific drug development.

Thomas Metcalfe from Roche Molecular Diagnostics (http://www.roche-diagnostics.com) predicted that theranostics products would capture half of the PGx market in 2013 and drugs that are currently low in efficacy would benefit most. Chris Chamberlain (Roche Products; http://www.roche.com) predicted the trend from low-efficacy high-responder phenomenon to a high-efficacy low-responder one. Albert Seymour from Pfizer (http://www.pfizer.com) believed that even slight improvement in predicting drug response would have great impact on a large population by reducing the number of patients required in clinical studies.

Typically, the integration of PGx in the drug development strategy starts with testing the potential pharmacodynamics biomarkers in Phase II trials and validating select ones in Phase III in which clinical outcome will be correlated with biomarker detection [Nicholas Dracopoli and Lukas Amler, Bristol-Myers Squibb (BMS; http://www.bms.com); Jeff Ross, Millennium (http://www.mlmn.com); Hakon Haonarson, deCode Genetics (http://www.decodegenetics.com). The testing of pharmacokinetics biomarkers is, however, best implemented in Phase I trials when wider range of drug doses are used on patients, as stated by Brian Spear from Abbott Laboratories (http://abbott.com).

Commercialization of PGx products

Several issues challenging the successful commercialization of PGx tests in the future ignited debates and optimism. Jorge Leon from Leomics Consulting dissected the multiple steps to PGx commercialization and product valuation. The key criterion for success is that PGx tests must enable clinicians' therapeutic decisions that existing diagnostics tests can not. Despite this enabling advantage, Gregory Tsongalis (Hartford Hospital; http://www.harthosp.org) believed the cost of new PGx tests needs to be low enough, and the logistics simple enough, for realistic adoption at the bedside.

Anne Bailey (Nuvelo; http://www.nuvelo.com) identified three critical disease areas, cancer, psychosis/depression and obesity, as having high incentives for PGx paradigm shift based on motivation of clinicians and patients. Another criterion for success is meeting regulatory requirements, as presented by Matthew Klamrzynski from Abbott and Karen Long from Roche Diagnostics. Currently, drug developers are granted 'safe harbor' for submitting PGx data under active IND while such data will not undergo formal regulatory review [3]. Long highlighted a new category of 'combination products' for submission and advised early discussion with the FDA (http://www.fda.gov) to determine the correct submission for review of the companion diagnostics. Education of FDA and the public on PGx tools and data interpretation was predicted to be a major cost in the PGx development, according to Metcalfe and also Klaus Lindpaintner (Hoffman La Roche).

Reimbursement of PGx tests by insurance payers represents the most provocative challenge for a reality check. David Parker (Covance Health Economics and Outcome Services; http://www.covance.com) believed that standardization of PGx tests is key to making pricing and, therefore, reimbursement possible. Significant restructuring of the current coding system will be needed to accommodate the testing of specific PGx biomarkers. Most discussion panelists agreed that a major change in reimbursement policy would eventually be driven by the significant breakthrough in drug treatment enabled by PGx.

Genotyping for ADRs and enhanced drug efficacy

Less than a hundred recent IND and NDA filings have PGx integrated in early drug development and genotyping is the predominant approach [3]. Brian Spear from Abbott reported that 30–40% current drugs have genetic components that determine their response in patients. Adverse drug reactions (ADR) are relatively rare and drug withdrawal cases are anecdotal, therefore, the PGx tests for ADR are most useful in determining the optimal efficacious and toxicity-tolerant dosing for individual patients (Lindpaintner). Common genes affecting pharmacokinetics, such as phase I drug metabolizing enzymes (DME) like cytochrome p450 (CYP) genes and phase II DME like thiopurine S-methyltransferase (TPMT) have extensive polymorphisms [4,5]. Examples of potential DNA-based tests based on single nucleotide polymorphisms (SNPs) included CYP2C9 for Warfarin (Metcalfe and Spear), CYP2D6 for psychiatric drugs (Lindpaintner), and UDP-glucuronosyltransferase UGT1A6 for Tasmir (Chamberlain).

Bailey upheld the importance of haplotype testing by explaining how patients with different haplotypes might be scored with the same multitude of genotypes without differentiation if genetic linkage is not determined, and how validated haplotyping will simplify statistical analysis by reducing the number of genes and size of patient samples. It is thought that accidental correlations

between disease phenotype and the estimated 30 million SNPs in the human genome will give rise to too many false positives [6]. A current limitation is the lack of simple haplotyping technology that measures multiple SNPs over long distances [7].

Stacey Gabriel from the Whitehead Institute (http://www.wi.mit.edu) gave an update on the Haplotype Mapping Project,and David Barker (Illumina; http://www.illumina.com) described the use of the high-density fiber optic bead array in the discovery of haplotype blocks. By contrast, Brad Margus (Perlegen; http://www.perlegen.com) preferred to use large-scale free SNPs determined by whole-genome scanning on a single chip to correlate with drug response.

From gene expression profiles to predictive biomarkers

Global analysis of gene expression using high-density GeneChip™ Arrays is a common starting theme in identifying predictive biomarkers for drug response or toxicity. The reliability of detecting half-fold or less changes in gene expression can be enhanced by error training with Rosetta software, according to Stephen Friend from Merck (http://www.merck.com), or using large sample size, as presented by Doug Dolginow from Gene Logic (http://www.genelogic.com). Once the marker genes are identified, separate clinical assays will be developed for scoring.

Ross reported the first integration of PGx into clinical development of the anti-cancer drug, VELCADE™, a proteosome inhibitor recently approved for treating multiple myeloma. Upregulation of marker genes involved in apoptosis were correlated with drug response, whereas some non-responder marker genes were associated with cell proliferation and survival functions.

Lukas Amler (BMS) presented two drug discovery projects where the correlation of gene expression profiles in well-characterized breast, lung or colon cancer cell lines with their IC_{50} profiles of drugs had led to the discovery of surrogate marker genes for sensitivity or resistance to EGF receptor kinase inhibitor or non-receptor kinase inhibitor. The screening of colon cancer samples revealed bimodal expression of the sensitivity and resistance marker genes. An immunohistochemistry assay has been developed for testing a high priority marker gene-product in the upcoming Phase II trials and validation in Phase III.

Proteomics approaches in biomarker discovery

Direct LC–MS analysis of clinical serum samples or other biologic fluids is the primary approach to discover metabolite, peptide or protein biomarkers associated with disease. This approach has unique practical value when disease tissues are not readily accessible for procurement. Howard Schulman (Surromed; http://www.surromed.com) believed that reproducibility is most crucial in identifying biomarkers among thousands of MS peaks and equipped his facility with quality assurance system. He presented a marker identified among 35,000 peptide differences in a rheumatoid arthritis model.

Stanley Hefta from BMS has identified 23 proteins that segregate toxic compounds from non-toxic compounds in a hepatic injury model using a similar approach. One biomarker was found to correlate with serum indications of hepatic damage in human.

Focused systems biology is another approach that enables the identification of protein markers that are associated with the pathology of diseased tissues or are potentially predictive of the activity of drugs based on their downstream location in the target signaling pathway. Po-Ying Chan-Hui (ACLARA Biosciences; http://www.aclara.com) described in a poster the use of proprietary multiplexed receptor dimerization, protein phosphorylation and gene expression assays to monitor the activation of EGF receptor family members and differential downstream signaling pathways in breast cancer cell lines. Potential biomarkers that differentially segregate breast tumors from normal tissues were presented.

Concluding remarks

It is becoming clear that the co-development of companion diagnostics to stratify patients for specific drug treatment, based on genetic predisposition for ADR and sensitivity or resistance to drugs, will lead to a new paradigm of supplementing traditional biochemical diagnostics with molecular diagnostics. The direct analysis of the diseased tissues will also enable rational selection of drug treatment to specifically address the pathology of the individual disease cases. It is the belief of most participants at the joint conference that PGx will change the practice of medicine.

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