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Identification of sequence variants or detection of contaminants, as different as viruses in human cells or bacteria in sludge samples of wastewater treatment plants, are some of the practical applications of PNA-chips. Simultaneously, the basic understanding of PNA–DNA interaction is being analyzed, information that could be critical also to very different fields such as antisense strategies.
Combining representational difference analysis with chip-based measurements, we are developing a methodology that should allow the quantitative study of transcriptional differences even between individual cells. These technical aspects, as with many of the above-mentioned, will improve the reliability of chip-based assays significantly to a degree to which the use of chips will be possible on a routine basis for many different areas including medical applications, for which quality standards have to be especially high.

Gene Discovery in Agriculture Research

Andres Binder
Novartis Crop Protection AG, Basel, Switzerland

The fast progress in modern biology represents a challenge for both pharmaceutical and agriculture research. New technology platforms like genomics, expression profiling, and bioinformatics open new dimensions in the discovery of genes, their regulation and functions. The unique advantage in agriculture research is to use gene discovery for novel solutions of crop improvements by the genetic as well as the chemical route: i) genetics: engineering and breeding of new germplasm (seeds) for crop varieties with novel genetic traits; ii) chemistry: development of novel chemicals through target-based discovery and high-throughput screening to protect crops against diseases, pests, and weeds, similar to drug discovery in the pharmaceutical field. Beyond this, the technologies also open up opportunities for new innovative solutions that combine chemical and genetic methods.
Besides human health care, one of the major challenges in science today is the improvement of our crop plants for food and feed production worldwide (rice, wheat, corn, vegetables *etc.*). This can be achieved either by improving traditional input traits for optimal plant growth using genetic and chemical technologies (*e.g.* protection against pests, diseases, and weeds), or – even more important in the future – by expressing new output traits in the plant with attractive benefits for the consumer, using gene technology (*e.g.* improved feed and food quality, medical and industrial products, processing efficiency, fiber quality *etc.*). The modern way to unravel these new traits is a gene discovery approach with the help of genomic technologies.
The basis for efficient discovery and comparison of genes and regulatory elements will be the availability of DNA sequences of relevant model systems and target organisms. Unlike pharmaceuticals research, agriculture research needs to cover many diverse organisms, *i.e.* sequence information of all the important crops, pathogens, and pests is needed. In addition, modern profiling technologies on various levels (RNA, proteins, and metabolites) are important tools to define the genes which are relevant for the expression of new traits in crops or represent new targets for novel agrochemicals.
The selected genes or regulatory elements for new traits can either be introduced in crops through plant transformation (GMO: introduction of foreign genes or change of regulation of endogenous genes) or alternatively they serve in a non-GMO approach as molecular information for marker-assisted breeding; the new efficient high-throughput breeding technology that exploits natural genetic diversity. For target-based discovery of novel agrochemicals (herbicides, fungicides, insecticides), potential targets from plants, fungi, or insects are first validated by inhibiting protein expression through knocking out genes with suitable methods (depending on the organism: homologues recombination, antisense, mutation tagging *etc.*) and by doing so mimicking the inhibition of agrochemicals. Knockouts giving rise to lethal or impaired phenotypes represent validated targets and will be used to develop assays for high-throughput screening (HTS) of thousands of new chemicals in minute amounts.
In this process from gene discovery to new traits or to novel agrochemicals, many centers of excellence are involved in a complex network: academic institutes with relevant biological systems, biotechnology boutiques with specific front-end technologies, companies with fully automated high-throughput machinery and potent international life science concerns with the breadth to coordinate and funnel the products into breeding and chemical development, worldwide field testing, and marketing.

Representative literature for this area:
- B. Mazur, E. Krebbers, S. Tingey, *Science* **1999**, *285*, 372–375.
- D. DellaPenna, *Science* **1999**, *285*, 275–279.
- C. Sommerville, S. Somerville, *Science*, **1999**, *285*, 380–383.
- E. Ward, P. Bernasconi, *Nat. Biotechnol.* **1999**, *17*, 618–619.
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B. Molecular Diagnostics (Chairman Prof. C. Weissmann)

Modern molecular technologies have revolutionized diagnostics. Five distinguished speakers presented recent advances in this field.
DNA amplification by polymerase chain reaction (PCR: T. White) and the analysis of DNA hybridization with miniaturized high density arrays of oligonucleotide probes (chip technology: T. Gingeras) became powerful techniques for the detection of DNA sequences or m-RNA expression *e.g.* for the diagnosis of genetic diseases or for the identification of bacteria or viruses. Proteomics, on the other hand, deals with the separation and characterization of proteins in biological samples, affords the identification of disease specific proteins, and offers opportunities for the discovery and development of new drugs (R. Parekh). Fluorescent coincidence spectroscopy (FCS) is a powerful technique for the rapid detection of molecules at extremely low concentrations, including viruses, amyloid peptides, ligand receptor complexes, and PCR products (R. Rigler). The mapping of single-nucleotide polymorphisms (SNPs), a frequent type of variation in the human genome, is a useful tool in pharmacogenetics (K. Lindpaintner). Abstracts of the lectures are given below.

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Recent Developments in PCR Technology

Thomas White
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In 1985 the first article on the polymerase chain reaction (PCR) method was published in Science [1]. In 1988, the first thermalcyclers and the Taq DNA polymerase became available for molecular biology research, and in 1992 the first diagnostic kits were sold for chlamydia and HIV. Although the initial impact of PCR on research and medicine was rather modest, today, 14 years after the Science paper, PCR is a technology worth more than 1 billion Swiss Francs, distributed about 20% in research, 10% in instruments such as thermalcyclers, 20% in diagnostic services, and 50% in diagnostic products. For diagnostic products, only ten pathogens account for more than 95% of the current testing volume, and also for most of the testing offered by diagnostic service (homebrew) protocols. Only a few genetic tests (cystic fibrosis and Factor V Leiden) are done in significant volumes.

PCR combined three known technologies (DNA synthesis, hybridization, and primer extension by a DNA polymerase) in a novel way. Today, the five surviving amplification methods (PCR, LCR, NASBA/TMA, SDA, and bDNA) also mostly combine other known techniques (*e.g.* ligation or reverse transcription) with the PCR concept of exponential amplification. The main focus today of diagnostics manufacturers is on automation, usually of detection first, then amplification and detection, and finally specimen extraction. A key aspect of this transition to automation relies on inventions which combine PCR with detection, such as the 5' exonuclease method invented by Gelfand, Holland, Saiki and Watson [2], which underlies Taqman™ products. Some of the advantages of these homogeneous amplification/detection methods include a broader dynamic range, higher throughput, lower risk of contamination, *etc.* Although most testing today focuses on detection, identification, and quantification, in the next few years new tests will provide additional information of medical value, such as antiviral drug resistance profiles and genetic factors which predict treatment responders from treatment failures.

Major future opportunities for PCR diagnosis are in genetics and cancer by means of prediction of disease risk, differential diagnosis, selection of therapy and dosage, avoidance of adverse effects, and monitoring drug efficacy and patient compliance. The practicality of diagnostic genetic tests can be compared with the experience with genetic tests for forensic and tissue transplantation over the last eight years, where over 1.8 million such PCR-based tests have been performed. The current phase involves multilocus genotyping assays and formats such as reverse dot blot strips and DNA chips, for common diseases such as arthritis and stroke. Further, mRNA profiling techniques, whether based on expression chips [3] or kinetic thermalcycling approaches [4], will permit high resolution analyses of patterns of gene expression which will be diagnostic for various cancers and/or allow identification of new targets for drug discovery and the prediction of drug efficacy.

[1] R.K. Saiki, S. Scharf, F. Falcoona, K.B. Mullis, G.T. Horn, H.A. Ehrlich, N. Arnheim, *Science* **1985**, *230*, 1350–1354.
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Proteomics as a Means of Discovering New Protein Targets and Bio-Markers of Human Disease

Raj. B. Parekh
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The molecular pathogenesis of most human diseases involves complex and multi-factorial processes. For many diseases the molecular abnormalities from which the disease process is initiated may commence years before a patient presents with that disease. Treatment can then only be achieved by modifying the sequelae of these causal events, *i.e.* by attempting to control or modify pathogenic processes present in a patient at the time of presentation. Since these pathogenic processes almost invariably involve aberrant protein expression, the discovery of disease-specific proteins as targets for modulating disease modifying processes is central to the discovery of new, improved drugs.

It is also true that the development of new drugs is substantially enhanced when objective differential diagnosis of disease can be achieved together with objective evaluation and measurement of disease. Patient management is improved when these diagnoses can be accurately performed early in disease. Since most diseases are associated with changes in the repertoire of proteins secreted by disease tissues, the discovery of new protein bio-markers has considerable potential to change for the better the diagnosis and management of human disease.

For these reasons, proteomics is a technology with enormous applicability to the discovery, development, and delivery of new drugs. The technology as presently applied involves a systematic analysis of expressed proteins from 'control' and 'disease' samples – as such it avoids many of the ambiguities of mRNA-based approaches and, by focussing on primary human material, the ambiguities inherent in analyzing animal models of human disease. This presentation discussed the relevance and importance of proteomics in modern drug discovery and development, the considerable technological advance that makes modern proteomics possible, and in particular its application to CNS disorders.

Use of High-Density Oligonucleotide Arrays in the Study of Infectious Disease

Thomas R. Gingeras
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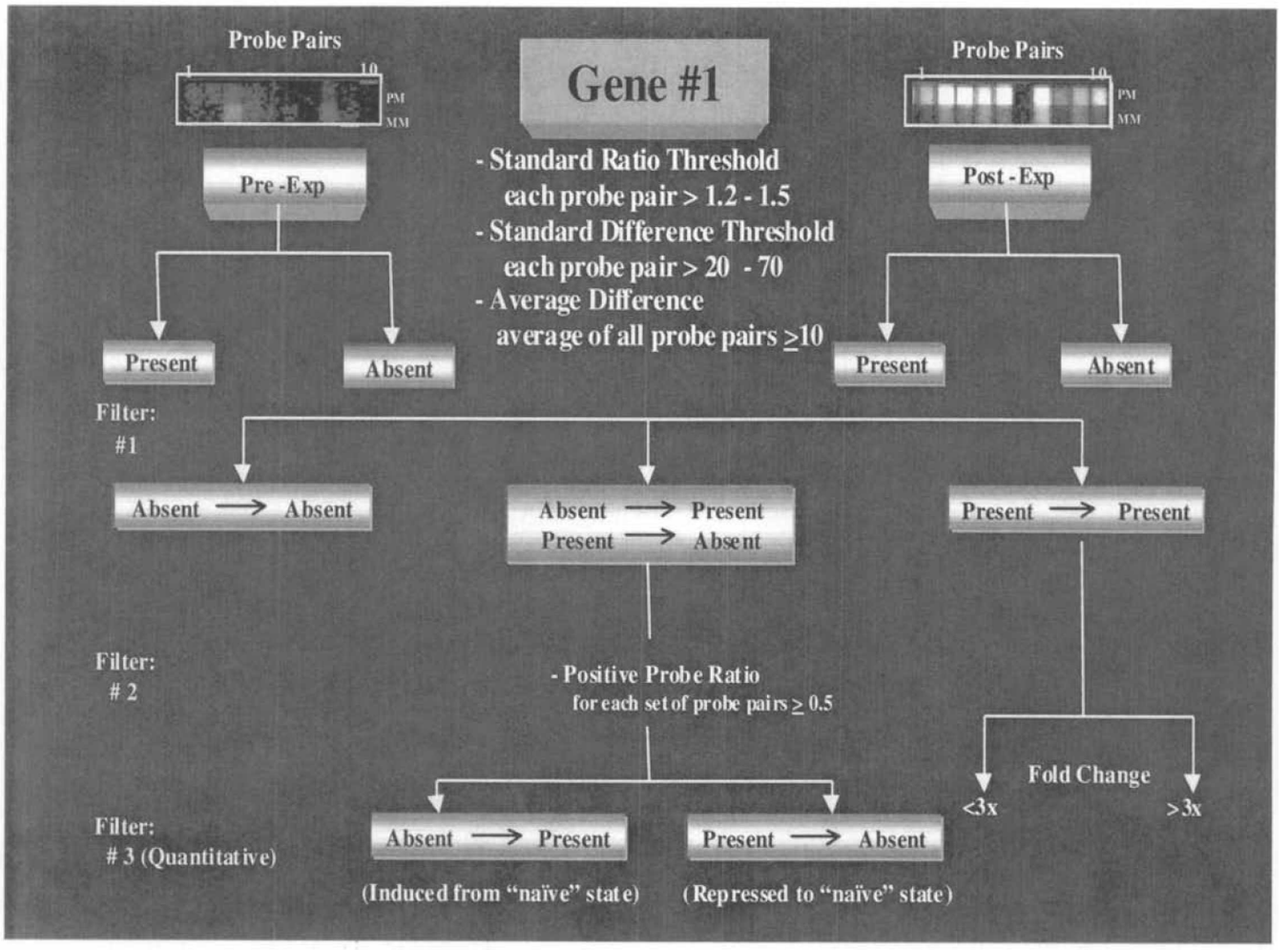
New technologies permit new types of experiments. The development of high-density oligonucleotide arrays has made it possible to monitor simultaneously and on an unprecedented scale the mRNA expression of thousands of genes in a cell [1]. The magnitude of this survey begins to provide a comprehensive understanding of the coordinated interaction of genes in the genome at the transcriptional level.

High-density oliognucleotide arrays have been applied to the analysis of mRNA expression in human, murine, yeast, rat, and bacterial cells (see bibliography at *www.affymetrix.com*).These types of discovery-driven research contrast with the more traditional hypothesis-driven experimental approach common in most molecular biology research. These two approaches are,



however, very complementary. However, critical to the efficient use and understanding of the data derived from this technologic approach is the ability to discriminate between good and poor data, as well as a means of organizing/interpreting the results so as to uncover new relationships and to confirm previously characterized ones.

An example which illustrates the issues of data quality and organization/interpretation, was to review the experiments conducted in collaboration with Drs. T. Shenk and H. Zhu in studying the temporal alterations in the mRNA expression of primary human fibroblast cells infected with human cytomegalovirus (HCMV) [2]. This study involved the infection of confluent primary foreskin fibroblast cells by HCMV strains AD169 and Toledo at a multiplicity of infection of 3. RNA was collected from infected cells at 0.6, 8, and 24 h after infection and analyzed using an array that interrogates approximately 6800 human genes. A total of 20 oligonucleotide probe pairs per gene is employed to derive a qualitative (gene is on/off) and quantitative (amount increased/decreased/unchanged) result. The use of multiple oligonucleotide probes in these arrays provides several advantages over the use of a single probe per gene-based arrays (derived from a cDNA clone or PCR fragment). Such multiple probes allow for 1) the well-documented specificity of oligonucleotides to be exploited, 2) significant redundancy of the interrogations performed on each gene, 3) the capability to detect and distinguish closely related genes belonging to a structurally related gene-family or splice variants and 4) the flexibility to synthesize all of the interrogating probes by a photochemical process which has been scaled to meet the demands of large numbers of interrogations.

The quality of the data produced in the HCMV experiments was evaluated by several criteria. Two of the important criteria were to determine that there was cDNA synthesis of sufficient length produced during the sample preparation steps to allow all of the probes selected for each gene to interact with their respective targets and that the reproducibility between replicate experiments allowed for comparison among time points. The cDNA synthesis integrity was evaluated using probe sets that interrogated the 5', middle, and 3' end of several constitutively expressed genes (*e.g.* actin, GAPDH) and the reproducibility of the replicate experiments conducted for each time point was evaluated using an analysis of variance algorithm.

Analysis of three time points occurring early in the infection of HCMV performed in duplicate for approximately 6800 genes produced more than 108 000 data points. Organization and interpretation of these data required a systematic approach. One approach is depicted in the Figure.

This scheme describes the use of three array-derived parameters (standard ratio, standard difference, average difference values) as a measure to categorize each gene as present or absent in each experiment (Filter #1). Having classified genes as to their expression state, Filter #2 describes a pooling strategy useful in the comparison of pairwise experiments. Based on the determinations carried out in Filter #1, Filter #2 describes a pooling strategy in which genes are clustered into groups which reflect the *type* of change in expression observed in the pre- *versus* post-

state (*e.g.* infected *vs.* uninfected). Only those genes observed present in both pre- and post-states are useful in determining the fold of the change. Those genes which changed their expression status from absent to present or *vice versa* should be viewed as demonstrating an 'infinite' change in gene expression and thus fold change calculations are not meaningful for this group. Filter #3 describes minimum fold change criteria that can be used to further classify those genes in the present to present group. The minimum fold change selected for this filter is determined by the variation observed between the replicates. This model of data organization and interpretation allows for the selection of alternative criteria used at each filter step as well as a straightforward means of evaluating gene responses in the pre- and post-state on a global level. In the HCMV infection experiments approximately 50% (>3000) of the genes analyzed on the arrays were observed to be present at each of the time points studied. Of these genes called present, the HCMV-AD169 infection demonstrated a total of 17, 60, and 167 genes decreasing threefold or greater in their expression at 0.6, 8, and 24 h, respectively and a total of 10, 33, 197 genes increased threefold or greater in their expression at each of the monitored time points. Similar numbers of genes were observed to change their expression levels for the HCMV-Toledo infection. Identification of the genes that altered their expression pattern threefold or more indicated that alpha/beta interferon regulated genes were, as a class, the most striking. One group of note in this class was the 2'–5' oligo A synthetase family of genes. Careful analysis of the data permitted the detection of differential expression of four splice variants of the mRNA of this family, thus illustrating the utility of having multiple oligonucleotides interrogate each mRNA in the expressed populations of messenger RNA. Finally, the consistent suppression or elevation of several genes in each of the infection experiments highlighted these genes for further analysis. One of these genes was microphthalmia transcription factor (MITF). Review of the literature indicated mutation in MITF was correlated with a loss of hearing phenotype in mouse and humans. This observation prompts the possible association of suppression of expression of this gene as being involved in the loss of hearing observed in infants born to acutely HCMV mothers. Such hypotheses serve to demonstrate the possibility of how results derived from high-density array experiments can serve as stimuli for new avenues of investigation.

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Applications of Fluorescence Correlation Spectroscopy in Diagnostics

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Fluorescence Correlation Spectroscopy (FCS) is based on the detection of the thermodynamic fluctuations of single molecules excited to fluorescence in extremely small volume elements (0.fl). From the correlated signal of the molecular noise, the actual number of molecules as well as their dynamic behavior can be obtained [1][2]. With its sensitivity (single molecule detection) and spatial resolution (submicron resolution) FCS offers unique properties when coupled to the high specificity of biological systems.

FCS has been applied to a series of molecular interactions including primer DNA targets [3], and has opened the field for nucleic acid diagnostics at the single molecule level [4]. The use of two-color cross correlations provides the possibility to follow PCR amplifications without any separation steps [5]. The aggregation process of Alzheimer ab proteins has been followed [6] and offers the basis for effective drug screening as well as clinical diagnostics. In a recent study performed at the cellular level, the existence of a highly specific receptor for the proinsulin-derived C-peptide could be demonstrated [7].

FCS is playing an increasing role in high-throughput screening given its sensitivity (single molecule), fast analysis time (subsecond), and small analysis volumes (submicroliter). An account of the development of FCS and its application in molecular analysis and diagnostics is in preparation [8].

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Genetics and Genomics: Aspects of Drug Discovery and Drug Development

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Two important developments characterized the past few decades of medical and pharmaceutical research. First, the advent of powerful molecular biology techniques allowed an understanding of cellular mechanisms that was previously not possible, reaching down to the level of the genome, and culminating in the unraveling of disease genes for rare monogenic diseases. This provides medical sciences with a new mechanistic understanding of biology and of the cause of disease, and holds the promise of providing us with a better, expanded toolkit for disease risk assessment and prediction, and thus the potential for preventive health care that had previously been the domain of classical epidemiology.

In parallel, and as a consequence of these developments, the pharmaceutical industry experienced during this time the passing of the baton from the chemist to the biologist: where chemistry once played the leading role, handing the biologist newly-forged compounds for testing in a variety of disease models, physiological mechanisms and disease models are now being stripped down by the biologist to reveal the critical targets for which chemists synthesize their molecules in a target-driven approach.

Lately, these developments have begun to include genetics on the level of drug discovery and development for those common

diseases that play the most important role from the standpoint of public health, which cause most human suffering and cost society most dearly. Logically so, as we know that heritability plays an important role in ailments that range from high blood pressure to diabetes, from rheumatism to asthma: they all show, in addition to their dependence on well-studied external factors, a propensity to cluster in certain families; and for many of these diseases a positive family history has long been recognized as one of the most significant risk factors. The elucidation of these factors surely should provide us with useful information on new, causative disease targets.

In addition, in as much as we have made important progress in developing effective and powerful medicines over the last decades, most of them remain fraught with unpredictable and far from perfect efficacy; and almost all of them also cause adverse often, unpredictable effects. Given that in many instances no clinical parameters have emerged as useful for predicting efficacy or side effects, one may speculate that different genetic predisposition – based on differential disease causation on the molecular level resulting in identical phenotypic manifestations, or based on differential metabolic handling of a drug molecule – may explain some of these differences.

It is along these lines of reasoning that we envision the impact of molecular genetics on the pharmaceutical industry. Some of these aspects will not be realized for some time to come, others may materialize earlier.

Thus, in the long run, the discovery of new drug targets based on the detection of novel disease mechanisms with the help of genetic investigations holds major promise for future, better drugs: because genetic studies provide causative targets – in contradistinction to most other medical research that is only associative in nature – they may serve to give rise to more effective drugs, at least for the subgroup of patients with a certain disease in whom the targeted mechanism is indeed causative. In as much as not all thus-derived targets will turn out to be chemically tractable, their identification opens the door to the discovery of other, linked elements of the same pathway or pathomechanism, among them perhaps more feasible molecules for drug targeting. Research into the genetic roots of common, complex disease has long focused on non-parametric approaches; the lessons learned have been mostly disappointing, and once again collection of large and informative families and pedigrees seems to become the preferred approach. In any event, the impact of newly found disease genes is envisioned to take considerable time, in as much as the expected higher success rate – based on the targeting of causal mechanisms – may improve the overall success rate and thus, decrease ultimately the average time invested until a drug reaches the market.

Once a drug target is selected, genetics and genomics offer – this time on a less far-flung timeframe – significant advantages for the compound discovery and optimization process: genetic epidemiology studies may help validate the target as not only one based on physiological understanding of and possibly therefore disease-associated mechanisms, but as one that may show – given a genetic variant exists and can be thus tested – association with the disease in case-control studies. Furthermore, any genetic variation – in most cases these will be single nucleotide polymorphisms, or SNPs, of a target – may indeed affect the binding characteristics of chemical compounds being synthesized in its pursuit; if this is the case in a target that, based on SNP analysis, shows no particular association with the diseased state, one may want to search for a ligand that is non-selective, having the same affinity for both molecular variants of the target molecule; conversely, if one were to show that only one variant is seen in patients with the disease, but not the other, then this variant would define a subcategory of individuals with the disease, and most likely the (best) responders – thus one might want to synthesize the most selective compound for the disease-associated variant of the target.

Genomic technologies, in particular high-throughput expression screening on the mRNA level, in the future almost certainly complemented by similar proteomics approaches, is poised to play an increasingly important role in compound selection – or compound rejection – at an early stage in the drug optimization process. Currently still hampered by the lack of availability of comprehensive data bases, toxicogenomics will thus help weed out compounds with potential long-term adverse effects based on the recognition of typical expression patterns; likewise, pharmacogenomic approaches will allow selection of the compound with an optimal spectrum of activity.

On an even shorter time frame, pharmacogenetic monitoring is expected to play a potentially major role in the understanding of and dealing with differential efficacy of drugs currently in clinical development. By saving a DNA sample from patients enrolled in phase 2 and phase 3 clinical trials, investigation and recognition of genotype-related differences in efficacy, as well as potentially in the occurrence of adverse effects, can be built into the clinical development process and may provide important early clues as to how a drug can be best used and marketed. In as much as we are all familiar with examples for this from the area of pharmacokinetics, systematic attention to this is likely to increasingly reveal similar inter-individual differences resulting in differential efficacy also on the level of pharmacodynamics.

Given the sensitivity – rightly or wrongly – with which any and all genetic research is met by the general public, it is imperative that anyone involved in these kinds of studies remains keenly aware of the important bio-ethical, social, and legal connotations of genetic research. Thus, proper attention must be paid to confidentiality and privacy issues, as well as to the patients' right to decide about the use of 'their' genetic material. In due course, we foresee the need for comprehensive legislation that will at least declare illegal all discriminatory and exploitative use of genetic information, thus enabling less emotionally burdened use of the information to the patients' benefit.

Although major progress has been made, and genetics has certainly crossed the threshold towards becoming a practical reality in the drug discovery and development process, much remains to be done. Ultimately, however, genetics, like any other major innovation in the biomedical sciences, will allow us to view the patient with better understanding, take a more differentiated approach, and treat – perhaps prevent – on a more individually optimized level.