Molecular genetic profiling

This was the topic of a Cambridge Healthtech conference held in San Diego on 5–6 May, 1997. The aim of the meeting was to examine molecular techniques for determining genetic profiles of individuals as well as patient populations. These profiles can be used for a range of purposes including patient stratification in clinical trials, as a guide to therapy, and for developing molecular diagnostics.

Technology development

The first part of the conference was devoted to technology development, and the key speaker was Dr John Snitsky (Roche Molecular Systems, Branchburg, NJ, USA). He discussed polymerase chain reaction (PCR)-assisted genetic profiling for infectious diseases and cancer to provide improved cost-effective health care. He pointed out the following challenges for routine diagnostic testing for patient stratification:

- correlation of disease phenotypes and treatment efficacy with pathogens and genes;
- technological developments for providing significant predictive value, high throughput and automation;
- definition of medical indications by strategically significant clinical trials; and
- timely approval of assays by regulatory authorities.

Dr Jorge Leon (Quest Diagnostics, Detroit, MI, USA) stated that genetic profiling of patients and micro-organisms will become an essential tool for patient management in the next century. He emphasized the need for improvements in data acquisition and integration of this information with patient clinical history and treatment options. Dr Laura Heisler (Third Wave Technologies, Madison, WI, USA) presented the technique of Cleavase Fragment Length Polymorphism (CFLP®), which involves scanning the whole length of the DNA fragment and fingerprinting both DNA strands. Primers can identify which bands come from which strands, and both strands can be analysed in a single reaction. Using the two-strand analysis method, the sensitivity is 98% for point mutations. This technique has been applied to the detection and localization of mutations associated with isoniazid resistance in *M. tuberculosis* and for differentiating bacterial genera, species and strains[1]. Dr Paul Siebert (ClonTech Laboratories, Palo Alto, CA, USA) showed that CapSwitch technology can be used for generating high-quality cDNA from sub-microgram quantities of RNA. This can be combined with suppression subtraction hybridization, a PCR-based method for cDNA subtraction.

Molecular diagnosis and profiling of cancer

The second part of the conference was devoted to molecular diagnosis and profiling of cancer. Dr Carol Dahl of the National Cancer Institute (Bethesda, MD, USA) described the Cancer Genome Anatomy project (CGAP). The purpose of CGAP is to enable early detection of cancer, to facilitate diagnosis and classification, assessment of prognosis and formulation of strategies for prevention of cancer. The goals are twofold:

- establishment of an index of all genes that are expressed in tumours (NCI tumour gene index) and
- development of technologies for high-throughput analysis of gene expression and mutation detection.

Dr Douglas Ross (Stanford University, CA, USA) reported on a method for comparative message expression for thousands of genes simultaneously by hybridization to PCR-amplified cDNA targets immobilized in microarrays on coated microscopic slides. This technique has been applied to gene expression profiling of cancer specimens. Dr T. Sekiya (National Cancer Centre, Japan) described a single-strand conformation polymorphisms method for detecting mutations in genes of which nucleotide sequences are known[2] and simultaneous hybridization of an arbitrarily primed PCR method for analysis of abnormalities in uncharacterized regions. Application of these methods to the analysis of cDNA from samples of human cancers has provided useful information on accumulated genetic abnormalities and progressed the understanding of human cancer. Dr M. Sherman (PharmaGenics, Allendale, NJ, USA) described the use of SAGE (serial analysis of gene expression) for assessing altered gene expression in human cancers. The main features of this technique are the use of short nucleotides to define transcription, efficient serial analysis, and that it is a quantitative method. A program for profiling of breast cancer was presented by Dr D. Dolginow (OncorMed, Gaithersburg, MD, USA). Use of GenChip™p53 (in collaboration with Affymetrix) was considered to have an advantage over sequence-based methods with 99% efficacy.

Clinical applications of genetic profiling

The final session was devoted to clinical applications of genetic profiling. Dr C.D. Earl (Avitech Diagnostics, Malvern, PA, USA) presented the technique of Enzymatic Mutation Detection™ for molecular genetic profiling in a managed care environment. This is a four-step assay involving amplification, hybridization, detection and analysis. Molecular genetic profiles can be generated rapidly and cost-effectively using advanced genetic profiling technologies. The role of the LifeSeq® database as a key tool in preclinical and clinical pharmaceutical research was described by Dr Susan Stuart (Incyte Pharmaceuticals, Palo Alto, CA, USA). The database has currently 326 libraries and a total of 1.8 million clones/sequences. The use of LifeChip™ (in collaboration with Affymetrix) has facilitated detection of gene expression and genome-wide mutation scanning. Dr S. Peroutka

  1359-6446/97/$17.00 PII: S1359-6446(97)01064-7

(Spectra Biomedical, Menlo Park, CA, USA) described Multiphenotypic Allelic Profiling (MAP) Technology™ as a rapid and cost-effective approach to developing diagnostic and therapeutic products. This technology involves sequencing of genes of about 100 receptors, channels or enzymes that are currently targeted by the pharmaceutical industry, in order to identify common polymorphisms. The Dopaminergic Allelic Profiling Program©, by performing a genotyping analysis, facilitates clinical trials for potentially novel indications: migraine, major depression, panic attacks and generalized anxiety disorder. The role of genetics in clinical development was reviewed by Dr P. Milos (Pfizer, Groton, CT, USA). Genotyping will be important in the design and interpretation of clinical studies. Identification of important human genetic polymorphisms can lead to a improved understanding of response to therapy. According to Dr G. Miller (Genzyme Genetics, Scottsdale, AZ, USA), molecular genetic profiling by systemic application of high-throughput molecular genetic analysis (MASDA™) would benefit clinical trials as follows:

- drug development time would be reduced by demonstration of therapeutic efficacy on responders;
- patients in clinical trials can be stratified according to their genetic profile, which would help in demonstrating the linkage between the subtypes and therapeutic efficacy and thus optimize therapeutic relevance;
- total time to market can be reduced as approval characteristics and specificity of who should take the drug is demonstrated;
- the chancing of approved payment in the marketing phase would be better if responders could be differentiated from non-responders.

The role of genotyping in clinical trials of Alzheimer's disease (AD) was reviewed by Mr Pierre Sévigny (Nova Molecular, USA). The presence of ApoE4 has been associated with a decreased response to Cognex[3], and ApoE4 genotyping is being used in clinical trials of several other acetylcholinesterase inhibitors. However, there is still controversy about the usefulness of genetic testing in AD trials, as was pointed out by Dr Larry Altstiel (Eli Lilly, Indianapolis, IN, USA). There is general agreement that ApoE is a risk factor for AD and that ApoE4 allele patients probably have a lower probability of positive response to drug than non-ApoE4 allele patients.

The conference participants were predominantly from the industry and this is where most of the work on this topic is being done. Overall, the standard of presentations was excellent and much new unpublished information was presented. I was left with the impression that molecular genetic profiling is indeed going to be an important part of drug development process in the future and several techniques are available.

REFERENCES

1 Brow, M.A.D. *et al.* (1996) *J Clin. Microbiol.* 34, 3129–3137
2 Sugano, K. *et al.* (1996) *Genes Chromosomes Cancer* 15, 157–164
3 Poirier, J. *et al.* (1995) *Proc. Natl Acad. Sci. U. S. A.* 92, 12260–12264
4 Jain, K.K. (1997) *Molecular Diagnostics* Decision Resources, Waltham, MA, USA

Prof. K.K. Jain
Jain PharmaBiotech
CH-4057 Basel, Switzerland
fax: +41 61 692 4461
e-mail: jain@bluewin.ch

In short ...

Wyeth-Ayerst has obtained worldwide rights to **3-Dimensional Pharmaceuticals**' (3DP; Exton, PA, USA) orally active thrombin inhibitors currently in preclinical development. The deal also gives Wyeth the rights to any follow-up compounds discovered through the collaboration. Wyeth will pay 3DP $64 million (including immediate cash, R&D funding and milestone payments) if its first product meets all milestones for three drug indications. Additional payments will be made if a second product is approved and 3DP will also receive royalties on sales.