- Make comparative claims versus competition
- Eliminate an important negative in existing products in the market
- Offer a higher quality product than those currently available
- Tap into current/emerging trends in society
- Offer a price advantage versus currently available alternatives

A drug could do with as many of these advantages as possible, but these are huge challenges. And superimposed on this is in the pharmaceutical industry is the necessity for rigorous clinical testing and the constraints of short effective patent lives.

Arlington has thrown down a challenge for some of today's leading authorities to address these issues at a special event – *Pharma R&Direction – Pharma 2005: An Industrial Revolution in R&D* – to be held in Barcelona in June (see *Diary* for contact details). 'Those companies which fail to restructure their R&D processes, track what they are doing, implement the right IT strategies and put the right skills in place will not survive in their current form' claims Arlington. The program will feature champions from big pharma, such as George Poste, and the biotech sector, such as Chris Evans. Let's hope the great men of R&D can show us the way.

*David Hughes*

# Pharmacokinetic and toxicology screening

Over the past decade, combinatorial chemistry techniques have led to a revolution in the practice of discovery chemistry. The number of active compounds entering the preclinical testing phase of a typical drug discovery project has increased by orders of magnitude. Pharmacokineticists and toxicologists now need to develop high-throughput technologies to screen this flood of compounds efficiently. These technical developments, and the growing importance of genomics and bioinformatics in preclinical drug development, were the twin themes of a conference on pharmacokinetic and toxicology screening that was held recently in London, UK (21–22 January 1998).

Oliver Flint (Bristol-Myers Squibb, Princeton, NJ, USA) set the scene, describing the difference between traditional and combinatorial methods of discovery chemistry. The combinatorial approach may easily produce >500 potential lead compounds in a single project. Flint summed up the 'high-throughput dilemma'. With increasing numbers of compounds to test, and no corresponding increase in resources, less information can be obtained about each compound. The objective of predictive toxicology screening is simply to estimate the risk of failure in development. The design of high-throughput toxicology screens, including the choice of cell types, endpoints and measurement techniques, should reflect this straightforward need.

## New technologies

New technologies for preclinical lead optimization that are now in use in the industry were described by several speakers, including Chris Atterwill (Roche Discovery, Welwyn, UK). Cytotoxicity screening, in which the measured endpoint is simply cell death, is relatively simple to scale up, but more complex mechanistic screens are likely to be more accurate. The pattern of gene expression changes under challenge by a toxic agent. Those genes that are upregulated under stress are termed 'stress genes', and their expression can be monitored if they are fused to easily detectable 'reporter' genes. Cells containing multiple copies of oligonucleotide probes for many different genes can now be placed on microarrays. The pattern of gene expression induced by a potential drug can then be measured by using these so-called 'gene chips'.

Another use of DNA array technology was described by Tim Gant (MRC Toxicology Unit, University of Leicester, UK). As gene expression is an arbiter of phenotype, it is possible to monitor the expression of genes involved in toxicological responses and so detect differential response to toxic challenges between cell types. Microarrays of oligonucelotides are now used to detect differences in gene expression between cell types and therefore to differentiate between more and less susceptible strains.


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Brian Burlinson (Glaxo Wellcome Research & Development, Stevenage, UK) described tools developed and used at Glaxo Wellcome to overcome the 'bottleneck' caused by high-throughput discovery chemistry. These include *in silico* systems such as the rule-based system DEREK (deductive estimation of risk from existing knowledge). Rules are based on the chemical properties of compounds. He also described several medium- and high-throughput *in vitro* screens, including tests for DNA damaging agents. One such assay, which uses gel electrophoresis to measure DNA migrating out of nucleosomes, is known as COMET because the damaged nucleosomes resemble schematic drawings of comets.

One of the most novel tests discussed used more traditional, low-throughput methodology. Clive Meredith [British Industrial Biotechnology Research Association (BIBRA), Carshalton, UK] presented the results of an EU-funded project to measure excitotoxicity in the nervous system. He and his European collaborators found that a delayed, sustained release of the *c-fos* proto-oncogene product correlated strongly with this toxicity. The role of this protein is unknown, although it is believed that it may be produced as part of a neuroprotective mechanism. Meredith has proved that it is possible to predict this toxicity by measuring the ratio of early to late *c-fos* production. In the current demonstration phase, this assay is being scaled up to test 100 compounds over two years – a contrast with the very high-throughput methods described by the speakers from industry.

## Genomics and gene expression

'Toxicologists are beginning to realize that to do toxicology without considering genomics and gene expression is like doing astronomy without a telescope' says Spencer Farr (Phase-1 Molecular Toxicology, Santa Fe, NM, USA). Interpretation of results from the DNA array technologies described certainly requires the use of complex bioinformatics and genomics tools. Farr described the contrasting deductive and inductive approaches to interpreting patterns of gene expression. The deductive approach relies on knowledge of protein function. By contrast, the inductive approach relies on comparing gene expression patterns in test compounds with those produced by well-characterized compounds. No knowledge of function is required. Farr gave a 'real-time' demonstration of this approach using the program ChemProfiler™.

Almost every biotechnology conference now features a presentation about structural bioinformatics. Mike Sternberg (Imperial Cancer Research Fund, London, UK) described the explosion, in the 1990s, of the numbers of protein structures determined. However, most proteins with known sequences still have unknown structures. The established technique of homology modelling is regularly used to model unknown protein structures based on those of evolutionarily related proteins. One example, of great importance in toxicology and pharmacokinetics, is the modelling of human cytochrome P450 variants. No structure of a human P450 has been determined experimentally, but every human variant has now been modelled from known structures of the bacterial enzymes. Drug substrates have been successfully 'docked' into the active site of a model of the 2D6 variant. Molecular modelling suggested that one acidic amino acid – Asp301 – would be important for binding drug molecules to this enzyme. This was later proved by site-directed mutagenesis. Sternberg also reviewed progress in predicting the structure and function of a protein from its sequence. It is currently possible to predict the fold or the function of at least half the protein products of a typical complete microbial genome.

## Adverse effects

A report published recently in the *Journal of the American Medical Association* stated that adverse drug reactions in hospitalized patients was the 4th–6th most common cause of death in the USA. Two speakers, Magnus Ingelman-Sundberg (Karolinska Institutet, Stockholm, Sweden) and Charles Crespi (Gentest, Woburn, MA, USA) described work relating to two important causes of adverse drug reactions: genetic differences in drug metabolism and reactions between drugs.

Approximately 40% of human P450-dependent drug metabolism is dependent on polymorphic enzyme variants, the most common of these being CYP2D6, CYP2C 9/19 and CYP2A6. These polymorphisms cause differences in the rates of drug metabolism. Patients who are poor metabolizers of a particular drug can experience an exaggerated response to the drug, or, conversely, a lack of response if a prodrug is not metabolized. By contrast, 'ultra-rapid metabolizers' will produce too much of a metabolite. Ingelman-Sundberg described differences in the proportions of P450 alleles that occur in particular ethnic groups. Some of these are benign, but others cause serious defects in drug metabolism. Drug companies now tend to drop compounds at an early stage if they are likely to be metabolized by polymorphic P450 variants. However, it will eventually be possible to prescribe individual drug treatment based on a patient's P450 genotype.

Considering the possible effects of even occasional adverse drug–drug interactions, Crespi cited the case of the heart drug Posicor®, which was withdrawn by Roche after a few patients died from adverse reactions with other medications. Many such reactions are caused by one drug inhibiting the P450 variant that metabolizes the second drug. This can lead to the concentration of the second drug building up to toxic

levels. Crespi described a high-throughput P450-inhibition screen, using fluorometric substrates, which has been developed by researchers at Gentest. It is only necessary to use a single substrate probe for each enzyme, because if one substrate of a particular enzyme is inhibited, all substrates of that enzyme will be.

### Under trial

Konrad Tomaszewski (Pfizer Central Research, Sandwich, UK) related pharmacokinetic and toxicity screening to clinical development by describing the factors affecting the choice of dose range for the first clinical trial of a potential drug: the so-called first-in-man (FIM) study. Generally, in such studies, single doses of escalating concentration are given to healthy male volunteers. Animal toxicity studies using high drug concentrations are used to determine the maximum dose given in this trial. In a typical paradigm, the dose is escalated until 10–20% of the highest tested concentration that showed no adverse effects in animals (the NOAEL) is reached. Tomaszewski suggested that, in some cases, it should be possible to progress to much higher concentrations in an FIM trial. Suitable candidates for this could be 'me-too' drugs of known mechanism, and cases where the adverse effects observed in animals are very minor or specific to the animal species used. He recommended a pragmatic approach to clinical trial design: 'FIM design should be based on science, not a calculator'.

This conclusion echoed a point made by Nigel Brown (Pheonix International Life Sciences, Montreal, Canada) in his keynote address. He said that, especially now that screening techniques can be almost fully automated, scientists working in this area need a broad understanding of the complete drug discovery process. Researchers are often too specialized. It would be useful to apply the 'horizontal' team based approach, which works well in small biotechnology companies, throughout the industry.

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# Overcoming HIV resistance

A novel protease inhibitor developed by US chemists could be effective against mutant strains of HIV that have developed resistance to currently used drugs, such as retrovir, and even combination therapies.

AIDS drugs disable HIV by inhibiting viral replication enzymes, such as protease. But, HIV mutates quickly and resistance to individual inhibitors can evolve within weeks. Combination therapy using reverse transcriptase and protease inhibitors together has so far remained a reasonably effective approach, although doubts about its long-term efficacy have being raised.

### Glimmer of hope

Chi-Huey Wong at The Scripps Research Institute (La Jolla, CA, USA) believes he now understands the mechanism behind HIV's rapid adaptation to current treatments. More than 45 distinct drug-resistant variants of HIV have been found in the past three years, according to Wong. 'We have studied the mutation patterns of HIV protease from patients who take the existing drugs and found that the enzyme often rejects the drug by reducing the size of the drug binding site', he explains. The mutant enzymes often exhibit cross-resistance to structurally distinct inhibitors, which means an alternative broad-ranging inhibitor is needed.

To exploit their findings the team looked at the corresponding binding site on current HIV protease inhibitors. They found that most of the drugs have a bulky group at the P3 position, which corresponds to the side chain of the third amino acid from the scissile bond of the substrate. This interacts with the constricted areas in drug-resistant proteases. They reasoned that reducing the size of the P3 group might lead to a new class of inhibitors that could still latch on to the HIV aspartyl protease even if a restricted region had evolved to block access of other drugs.

The team used molecular modelling techniques to look at the enzyme fit of alternative P3 substituents. They found that by modifying various known antivirals to have a methyl group at P3 instead of the usual bulky substituents endows them with a remarkably different pattern of inhibition, in the test tube at least. The team observed a 120–1000-fold improved inhibitory activity against HIV aspartyl protease and at least three orders of magnitude higher potency for feline immunodeficiency virus (FIV) aspartyl protease compared with the activity of the parent drugs. Modification of two existing drugs showed a similar effect, says Wong. The researchers also found that if there was no group at all at P3 (i.e. the group was replaced with

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