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Strategic and technical challenges for drug discovery

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The recent *Drug Discovery 2000* conference in Cannes, France, organized by Business Intelligence, attracted a blend of industrial and academic contributors, addressing the major issues and challenges facing the pharmaceutical industry in the new millennium. Presentations addressed the financial, strategic and technical imperatives of the changes in approaches to drug discovery, from target validation to drug development. Although many themes were discussed, prominence was given to the increasing recognition that drug metabolism and toxicity studies must be integrated into the discovery process.

Financial and strategic imperatives

Comments from Ian Skidmore (GlaxoSmithKline; GSK, Ware, UK) set the scene for many discussions as he outlined the demands that the stockmarket would place on big pharma, with the current requirement of one major drug launch per year, rising to 3–5 by 2005 and 6–10 by 2010. Skidmore highlighted the major challenges to modern drug discovery and the current trend for industrialization of these processes. The challenge concerning the trade-off between productivity and efficiency was discussed by many speakers.

The implications for discovering new drugs in the post-genomic era were addressed by William Koster (Bristol-Myers Squibb, Princeton, NJ, USA) and Chris Mundy (Medical Research Council, Cambridge, UK). Target validation for exploratory research is now aided by numerous technologies enabling gene expression, functional analysis and pathway discovery in whole-cell or animal models. Furthermore, the use of human expression systems including knockout or knockin mice to produce human homologues provides increased confidence in the legitimacy of the biological target.

Compound screening has likewise benefited from efficiency gains, from semi-automation in the mid-1990s through to full automation in the late-1990s to the current situation of ultra-HTS employing automated storage and retrieval (haystack) and miniaturization (Aurora Biosciences, San Diego, CA, USA) systems. Ever-increasing testing densities are anticipated for the future through chip-based technologies.

Integrating technologies

Jeremy Everett (Pfizer, Sandwich, UK) discussed the integration of lead discovery technologies. This had been achieved both through the development of proprietary in-house systems and through substantial external collaboration (with Evotec, Aurora, Neurogen and ArQule). The process described went from selection of compounds for screening, automation and coordination of the screening processes, through to library design and large-scale purification.

Rationalization of the Pfizer sample collection has led to compounds that are 'rule of 5'-compliant, pure, available and possessing no toxicophores. Subsequent additions are designed based on leads emanating from HTS and chemoinformatic design, which will consequently already possess drug-like properties. A large automated purification system for libraries is used consisting of preparative HPLC and off-line LC–MS analysis for compound verification. The sample collection and new libraries are both held in a cold store. Samples are then delivered through a defrost station to a liquid handling cell via a conveyor belt, where they are cloned before the daughter plate is screened and the mother plate returned to storage.

In silico tools

The increased use of *in silico* tools was described by several speakers. Stephen Pickett (Roche, Welwyn Garden City, UK) described how, as *in vitro* absorption and metabolic stability measures are now available to screen large numbers of compounds, such data might now be being used computationally to influence compound design and distinguish drug-like from non-drug-like compounds prior to synthesis. This technique is extended beyond simple ADME properties to encompass CNS penetration through the use of polar surface area measurements. Similarly, Ajay (National Institutes of Health, Bethesda, MD, USA) discussed some of the additional complexities in modelling blood–brain barrier (BBB) transport with the emerging knowledge of the involvement of influx and efflux transporters nullifying simple lipophilicity-based models. This approach of modelling compounds uses known marketed drugs as training sets categorized as CNS^+ or CNS^-, depending on their therapeutic classification. They found that, with such an approach, predictions were generally high (>80%), with the associated caveat that the model would generally most accurately predict structures that resemble existing drugs.

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Jens Sadowski (AstraZeneca, Mölndal, Sweden) gave an overview of the current state-of-the-art in predicting toxicity computationally. As cytotoxicity screens can serve as a surrogate of whole-animal toxicity, it was shown that ~80% of a test dataset could be accurately predicted using data training sets, so acting as a powerful filter for compound prioritization.

The usefulness of predictive *in silico* ADME models in drug discovery was outlined by Peter Eddershaw (GSK, Ware, UK). Models for absorption, the BBB penetration and metabolism prediction used routinely by the GSK discovery scientists were described. Additionally, more specialized *in silico* methods for cytochrome P450 (CYP450) binding affinity and site of metabolism were shown. These approaches have been used to aid library design and to highlight new chemical entities for *in vitro* and *in vivo* evaluation. Furthermore, they can be used to speed up the decision-making process by filtering out compounds with undesirable properties, and to aid the understanding of structure–property relationships.

A collaboration between GSK and Camitro (Menlo Park, CA, USA) for the prediction of metabolically labile sites exemplified the impact these tools can have on rational drug design. Based on these models, the medicinal chemists were able to minimize total numbers of unstable sites and, consequently, reduce metabolism rates. It was apparent that *in silico* approaches have had a profound impact on the effectiveness of drug discovery when used either to complement *in vitro* and *in vivo* methods or independently using virtual screening approaches.

Early ADME/tox studies

Martin Bayliss (GSK, Stevenage, UK) demonstrated how pharmacokinetic (PK) and metabolism studies can now be integrated into the lead optimization process. The traditional view of potency and selectivity is being superseded by approaches that balance these factors with suitable ADME/tox, as these factors are a major determinant of whether leads will become good medicines. Automated systems for *in vitro* metabolism or absorption screening enable many tens of compounds to be analysed in an 8-h period. This represents a substantial saving over manual methodologies (hours versus weeks), providing an effective filter for inappropriate properties. By utilizing a combination of *in vitro* assays and *in vivo* cassette dosing, 150 leads from HTS could be rapidly prioritized for further optimization.

Alan Watt (Merck, Sharpe and Dohme, Harlow, UK) took up the theme of higher-throughput PK. The mass spectrometry revolution of the past ten years has given drug metabolism groups the requisite analytical capabilities, shifting the bottleneck to sample preparation. It was shown that automated robotic sample preparation technologies utilizing 96-well plate technology had been designed to satisfy demanding tolerances of precision and accuracy for quantitative analysis. A fivefold improvement in assay time, with the majority unattended was reported, enabling provision of PK data in real time to project teams. Such technology was being extended to encompass the diversity of drug metabolism assays now required for discovery support. Furthermore, with the current and anticipated future reliance on mass spectrometry, Visual Basic (Microsoft) routines for the automated optimization of MS conditions and preparation of sample lists were described.

A comprehensive overview of discovery ADME was provided by Rashmi Barbhaiya (Bristol-Myers Squibb, Princeton, NJ, USA), who highlighted the multiple interactions of a discovery DMPK group, not only with medicinal chemists and biologists, but also in the more downstream activities of safety and pharmaceutics. The theme of 'fail fast, fail cheap' and the concept of compound developability were again emphasized with a balance of *in vitro and in vivo* assays, all available in a higher-throughput mode. Affinity versus metabolic turnover plots were used to select potent, stable leads and a screening cascade of CACO-2 permeability, metabolic stability and solubility has been developed to address oral PK issues. Attention was paid at an early stage to the potential for drug–drug interactions through CYP450 inhibition and isoform contribution studies, and an example where allometric scaling had been used to successfully predict the human PK for gatifloxacin was provided.

Gordon Gibson (University of Surrey, Guildford, UK) showed some of the latest developments in assessing human CYP3A4 induction in human systems using reporter gene assays. The role of the glucocorticoid and the pregnane-X receptors in regulation of CYP3A4 expression was discussed and evidence for CYP3A4 polymorphism was presented. A human liver pool (11 subjects) revealed a 14-fold spread in CYP3A4 activity by probe substrate phenotyping. Western-blotting demonstrated that poor metabolizers generally show lower CYP3A4 expression than extensive metabolizers. Using single-strand conformation polymorphism (SSCP) mutation screening, two separate single-point sequence mutations were found in the liver responsive element (HNF-5) and the oestrogen receptor element (ERE). These were shown to be functional in that HNF-5 mutation resulted in reduced induction of CYP3A4 in response to hydrocortisol and dexamethasone, and ERE mutation showed reduced induction against oestrogen. This is the first known demonstration of functional genetic polymorphism in CYP3A4.

Drug delivery issues

Peter Warne (Aventis, Essex, UK) discussed the problems arising from poor drug delivery and the negative consequences of this in terms of data inconsistency and the consequent cost of performing repeat clinical studies. Examples where poor solubility, variable particle size and

crystal polymorphisms impacted on programmes were discussed suggesting that salt selection is an activity that must be brought forward in the cascade, prior to regulatory toxicology.

Barry Hirst (University of Newcastle, Newcastle, UK) focused on *in vitro* models of barrier transport outlining the CACO-2 cell line for permeability measures as an aid to predicting oral bioavailability. This could be made suitable for higher-throughput discovery work using the BIOCOAT™ HTS 3-day culture systems (BD UK Ltd, Oxford, UK) and the Multiwell® insert plates (BD UK) for compatibility with automated culture feeding and robotic measurement. The emerging area of transporters, particularly P-glycoprotein-mediated apical efflux, was discussed, with a huge range of therapeutic classes (>15) known to be substrates, highlighting the importance of this field. The use of the MDCK cell line as a model of passive permeability was suggested as this has a low expression of transport systems, and while cloned transporter systems appear attractive, there is a risk of endogenous expression of confounding transporters.

Conclusions

In many respects, the conference was a timely reminder that the pace of change, particularly in drug discovery, has never been greater, and that integrating and refocusing traditional disciplines to match the new 'parallel' discovery paradigms will continue to challenge researchers in the coming years.