DDT Vol. 8, No. 4 February 2003

Smart high-throughput screening

Matthew Segall, ArQule, 127 Cambridge Science Park, Milton Road, Cambridge, UK CB4 0GD; tel 01223 706 152, fax: 01223 425 416, e-mail: mds21@phy.cam.ac.uk

High-Throughput Screening Technologies 2002 (26–27 November 2002, London, UK) addressed the use of 'smart HTS technologies' in all aspects of the drug discovery pipeline. Discussions covered three broad areas – high-content screening, improved approaches to lead identification and optimization, and uHTS.

High-content screening

There is currently a need for higher quality lead compounds to improve the productivity of R&D. David U'Pritchard (3-Dimensional Pharmaceuticals, http://www.3dp.com) described an approach that balances HTS with structure-based drug design to link the post-genomic 'science framework' of target families, cell biology and pathways, with the clinical 'therapeutics framework'. Based on the premise that high-energy binding sites for small molecules are likely to be functionally significant, combining affinity screening and co-structure x-ray crystallography provides a rapid approach to identifying drugable targets and gaining in-depth structural knowledge to guide compound design. He described the Thermoflour® assay, which provides a thermodynamic readout of ligand binding. This approach can provide a primary HTS affinity screen in systems that are difficult to assay, and a secondary mechanism of action screen that enables, for example, the identification of reversible and irreversible binding, or multiple binding events.

Nick Thomas (Amersham Biosciences, http://www.amershambiosciences.com) introduced smart cellular assays and described their use as both functional assays for target identification and validation and for secondary compound screening. A combination of fluorescence-based assays, image processing and automated analysis was described by Philip Hargreaves (Beckman Coulter, http://www.beckman.com/) to capture high-content data from a cellular screen. Using multi-channel fluorescence and multiplexing, quantitative data can be generated on the localization of fluorescent-labelled components in multiple functional phases.

Case studies of cell-based screening were presented by Angela Cacace (Bristol-Myers Squibb, http://www.bms.com) and Claudine Grepin (Aventis Pharma, http://www.aventis.com). A high-throughput approach to 'de-orphanizing' orphan G-protein-coupled receptors (oGPCRs) was described by Cacace, overcoming the challenge of validating a screen in the absence of a cognate ligand by leveraging existing GPCR assays. Grepin outlined two approaches: first, dual reporter gene assays, enabling simultaneous screening against two targets to reduce costs and improve compound specificity; and second, a homogenous time-resolved fluorescence (HTRF) technique enabling rapid development of relevant, robust and sensitive assays. Several of the cell-based screens described were based on the Acumen™ Explorer System (Acumen Biosciences, http://www.acumenbioscience.com), including cytotoxicity, surface-antigen recognition and cell spreading. In contrast to the imaging system described by Hargreaves, the Acumen™ Explorer System gathers datapoints along scan lines, enabling objects in the field to be reconstituted for analysis.

Moving a step beyond cell-based screens, Frank Strigglow (KeyNeurotek, http://www.keyneurotek.com) introduced TELOMICS™ – a technique for tissue-based functional screening for target identification and/or validation and preclinical drug development. Focusing on CNS applications, functional assays have been developed based on cultured cerebral tissue slices. These have the advantage of retaining the context of intercellular networks and enable efficient functional screening before *in vivo* experimentation.

Lead identification and optimization

Improvements to the hit-to-lead process were addressed by Ulf Boemer (Schering, http://www.schering.de), who described the creation of a dedicated hit-to-lead team to improve the quality of leads and reduce the time taken in the hit-to-lead process. A series of decision points were defined along with criteria for lead selection, including molecular properties, pharmacodynamics, pharmacokinetics and potential for further optimization. The process for generating compounds and data was defined and optimized, and a pilot study successful completed. Members of the hit-to-lead team now contribute to all projects during this phase.

Andrew Baxter (AstraZeneca, http://www.astrazeneca.com) presented a number of hit-to-lead case studies. In each case, the goal was to generate three lead series in six months, fulfilling a range of lead criteria, including potency, pharmacokinetics and physiochemical properties. Hit-to-lead

  PII: S1359-6446(03)02598-4

'traffic lights' are used to indicate whether a lead: (1) meets each lead criterion (green), (2) narrowly fails to meet a criterion (yellow), or (3) fails by a large margin (red). These are used to guide the decision to progress a potential lead.

Optimization of absorption, distribution, metabolism and elimination (ADME) properties guided by *in silico* models was described by Matthew Segall (ArQule, http://www.arqule.com). A consensus scoring scheme, combining predicted ADME properties to give a single score, enables compounds with an appropriate balance of properties to be chosen from large virtual libraries. Applications of ArQule's models and scoring scheme were demonstrated to select metabolically stable compounds in a series with a metabolic liability to cytochrome P450 3A4.

In silico predictions can be integrated with *in vitro* measurements in a physiologically based pharmacokinetic model to predict pharmacokinetic parameters such as plasma concentrations, fraction absorbed and volume of distribution. David Leahy (Cyprotex, http://www.cyprotex.co.uk) described the use of such a model in combination with models and high-throughput screens for ADME properties to identify key compound properties for pharmacokinetic optimization.

Virtual screening demands a high-performance computing platform with easy access for researchers. Richard Scott (De Novo Pharmaceuticals, http://www.denovopharma.com) described the provision of such a platform using Grid technology, enabling careful control of resource allocation and simple access through a web-based front end.

uHTS

The development of uHTS, from 96-well plates through to 384- and 1536-well plates, was summarized by Irvine Winkler (Aventis; http://www.aventis.com). Significant improvements in throughput can be achieved by moving from a 96- to 384-well format. However, the improvement is less significant when increasing from a 384- to 1536-well format, owing to a lack of 1536-well pipetting heads and the extra dilutions required for low-volume formats. Although moving to 1536-well plates saves significantly on reagents, equivalent savings can be made with a low-volume 384-well format. Günther Knebel (Greiner Bio-One, http://www.greiner-bio-one.com) described how optimization of well-shape in low-volume formats yields improved sensitivity, equivalent to 1536-well plates in the 2–20 μl range.

When working with low volumes, transfer of compound from neat DMSO stock while keeping DMSO concentration low and sample concentration high requires dispensing in the low nanolitre (nl) volume range. John Comley (a consultant in HTS technologies) expanded on the challenges of nl dispensing compared with current methods. Contact methods are the least expensive, although suffer from reliability issues and have a realistic lower limit of 250–500 nl. Non-contact methods, such as sensor-controlled solenoid valves, piezo-actuated- or capillary-filling-heads, offer higher reliability but are more expensive. True non-contact methods, such as acoustic actuation, offer the best flexibility and reliability but are not currently available. Comley also introduced the concept of the dispensing-well plate, which consists of reagent reservoirs coupled to nl dispensing nozzles filled by capillary action. This is currently under development by IMTEK (Institute for Microsystem Technology: http://www.imtek.uni-freiburg.de).

A contrasting perspective was offered by Mark Beggs (The Automation Partnership, http://www.automation-partnership.com) who argued that higher throughput could be achieved without the need for investment in new technology. Data from the modern manufacturing industry indicate that flexible dynamic scheduling and design of facilities using computer simulations can deliver facility performance in line with business objectives using current technologies.

Concluding remarks

The overall consensus from the conference was clear: simply undertaking more conventional HTS will not be sufficient to meet the needs of the modern pharmaceutical industry. Potential solutions to the challenge of generating higher quality leads for increasing numbers of targets were suggested that could facilitate the delivery of more new chemical entities to the market.