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HTS beyond the human genome

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As the number of druggable targets increases with the completion of the Human Genome Sequence, HTS strategy continues to take on a more significant role within the drug discovery process. This strategy is now reflected in the upstream and downstream processes of biomolecular screening, namely, target identification and lead optimization. This was evident at the *Society of Biomolecular Screening (SBS) Seventh Annual Conference and Exhibition* entitled *Beyond the Human Genome* (10–13 September 2001, Baltimore, MD, USA). The conference brought together 2800 attendees and 170 exhibitors from 29 countries; these included technology developers and discovery scientists representing disciplines from chemistry, biology, engineering, automation and informatics.

The sessions spanned four days and included: *Genomics, Proteomics and New Target Identification*, *Biosensors*, *Automation*, *Biochips*, *Nanorobotics*, *Compound Library Generation & Management*, *Assay Development and Validation Strategies*, *High-Throughput ADME and Toxicity Strategies*, and *Bioinformatics*. In addition to the oral presentations, short courses, and exhibitor workshops and tutorials, there were over 375 posters presented on a range of relevant topics.

Target identification and validation

As throughput increases and cost reductions continue in HTS, greater emphasis is placed on development and implementation of high throughput technologies as it applies to target identification and validation. This was the focus of the *Genomics, Proteomics and Target Identification* session. Highlights included the presentation, by Jonathan Rothberg (CuraGen Corporation, New Haven, CT, USA), of the successful application of genomic technologies and bioinformatics to discover novel drug targets. The identification of *in vivo* mammalian function of genes using the mouse knockout model at Deltagen (Redwood City, CA, USA) was described by Mark Moore. Christian Frisch presented the high throughput generation of antibodies for target validation using proprietary technologies developed at MorphoSys (Munich, Germany). The use of ThermoFluor, a thermal denaturation technology for target identification at 3-Dimensional Pharmaceuticals (Exton, PA, USA) was presented by Eugene Petrella.

Biochips

A keynote lecture given by George Whitesides (Harvard University, Cambridge, MA, USA) addressed the major advances and issues in the field of biodevices, biochips and microfabrication. Overall, Professor Whitesides believes the future of micromachined substrates for biotechnology will be plastic, and not silicon or glass.

As developed HTS chip and microtechnologies become adopted by discovery scientists, others emerge on the horizon. A few of the microtechnology highlights included a description of the use of either reporter genes or fluorescent indicators to monitor physiological and genetic responses of cells using a novel single-cell array technology, fabricated on optical imaging fibers (Israel Biran, Tufts University, Boston, MA, USA). Nathaniel Hertz (Eli Lilly, Research Triangle Park, NC, USA) shared the experiences of using a quad-sipper chip/instrument whereby 17,000 compounds could be screened per day, with costs that were comparable to a plate-based screen. This was performed by the adoption of microfluidic technology from Caliper Technologies (Mountain View, CA, USA). Development of a cytochrome P450 assay with the LabCD, a centrifugally driven microfluidic manifold, which showed comparable performance to data generated in a 96-well format, was described by Howard Haspel (Tecan, Mannedorf, Switzerland).

Technology comparisons

As HTS technologies evolve and new technologies emerge, they quite often converge, offering drug discovery scientists a choice. This was not only a focus of a dedicated session on comparison of technologies but was a recurring theme throughout the conference.

Numerous presentations focused on comparisons between detection, as well as reagent, systems. Most of these focused on the differences in sensitivity, throughput, cost, compound interference and quality of leads. Louis Stancato from Eli Lilly (Research Triangle Park, NC, USA) compared data from HTS for kinase inhibitors using radiometric, fluorescent and microfluidic approaches, and Jinzi Wu (Immunex, Seattle, WA, USA) discussed their evaluation of HTR-FRET and DELFIA for the identification of TNF inhibitors and how the choice of assay technology can affect the outcome of hits. In addition, David Cronk (GlaxoSmithKline, Stevenage, UK) and Paul Johnston (Eli Lilly, Research Triangle Park, NC, USA) compared how fluorescence assays using FLIPR® and VIPR™, both established platforms for kinetic cell-based screening for ion channels, have been used successfully in ion-channel drug discovery.

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Unfortunately, the last two days of the *SBS Conference* were blighted by the terrorist attacks in the USA. Despite the tragic events, the program continued, to highlight technological breakthroughs and innovative approaches in drug discovery, with the mantra being continuous improvement and use of tools for faster, more accurate data generation, analysis and interpretation.

Non-conventional assay approaches

To accommodate difficult targets and increase the efficiency of screening, scientists are developing non-conventional assay platforms. Seminars in this session described new assay systems, including affinity screening using MS–NMR, the use of yeasts and nematodes as tools for drug screening and determination of the mode of action of drugs via metabolomics.

In his plenary speech, Douglas Kell (University of Wales, Aberystwyth, Wales, UK) described functional genomics, the mode of compound action via metabolomics and machine learning, emphasizing the importance of investigating metabolic fingerprinting rather than the proteome or transcriptome to assess novel gene functions. The analytical methods for determining the metabolites are rapid, specific, reproducible and low cost. The multivariate data consists of observations on variables for a number of objects and the data analysis is by pattern recognition. This technique is very useful, not only to identify particular genes, but also to learn the pharmacological action of drugs.

A rapid screening paradigm using MS–NMR to detect the binding of small-molecule compounds to specific protein targets was reviewed by Robert Powers (Wyeth-Ayerst Research, Cambridge, MA, USA), where an MS–NMR screen is performed using mixtures of ten compounds. After incubation with the target protein, compound(s) that remain bound are separated by size exclusion chromatography and identified using MS and NMR. Data on the function of the target are not required, the assay provides structural information on specific binding events, and is a powerful method with high sensitivity and throughput. Juergen Soutschek of Devgen (Ghent-Zwijnaarde, Belgium) then spoke about using the nematode *Caenorhabditis elegans* as a model system for screening and identification of new lead compounds. The transparent worm provides the ability to screen most target classes and pathways and this system can measure precisely the activity of many chemical samples in a high-throughput mode.

John Swindle from Complegen (Seattle, WA, USA), a functional genomics and drug discovery company, described the use of specialized strains of *Saccharomyces cerevisiae* as a novel screening platform to identify new leads. The system is simple and versatile and can be used as a surrogate to survey the activity of targets from the genomes of humans, plants and other fungi.

Assay development and validation strategies

This session concentrated on G-protein-coupled receptors (GPCRs) and nuclear receptors, emphasizing the importance of these target classes in the drug discovery arena. GPCRs are the targets of >50% of current drugs on the market, including more than a quarter of the 100 top-selling drugs.

Richard Heyman (X-ceptor Therapeutics, San Diego, CA, USA) described the integration of orphan receptors as molecular targets for drug discovery. Recently, ligands for some of these receptors have been identified, providing recognition for the efforts of X-ceptor to develop a platform to identify ligands for orphan receptors for various diseases. Allison Chin (Geron Corporation, Menlo Park, CA, USA) discussed the use of embryonic stem cells for genomics and drug discovery, focussing on her experience of culturing these cells to form embryonic germ layers and the issues related to large-scale production of the cells for target identification and validation.

The application of enzyme fragment complementation (recombination of inactive fragments of *Escherichia coli* β-galactosidase to generate a functional enzyme) for target validation and lead identification, was presented by Richard Eglen (DiscoveRx, Fremont, CA, USA). He discussed the requisites of the enzyme donor and acceptor properties and their applications in measuring protein expression, exploiting cellular pathways and developing homogeneous assays for several target classes, including enzymes and receptors.

Robin Hyde-DeRuscher (Karo Bio, Durham, NC, USA) introduced their Biokey assay, which monitors GPCR activation by developing surrogate peptide ligands from phage-display peptide libraries. During the conformational change of the G-protein (GDP–GTP), the Biokey probe preferentially binds to the GTP-G_α subunit. Because the Biokey probes bind selectively to the functional site of the target protein and compete with small molecules, this technology can be formatted into an HTS assay and could also prove to be a good platform for screening all GPCRs, including orphan receptors.

Bioinformatics and data mining

The vast amount of data generated by HTS requires powerful data mining tools to extract useful information. The conventional means of dealing with data on an item-by-item basis are no longer sustainable. Christopher Keefer (GlaxoSmithKline, Research Triangle Park, NC, USA) discussed the MultiSCAM™ software, a novel application that is an extension of the recursive partitioning software, SCAM®. Matthew Hahn (SciTegic, San Diego, CA, USA) described their new approach to informatics, Data Pipelining™, which allows the processing, analysis and mining of large volumes of data via a user-defined

computational protocol. It also offers unlimited flexibility as it happens in real-time and can clean suspect data, merge data sources and perform real-time data calculation and reduction.

Biosensors

Although the concept of biosensor technology is >50 years old, it is starting to show its impact in HTS. Matthew Cooper (University of Cambridge, UK) presented the generic Biacore™ surface plasmon resonance (SPR) system and demonstrated its use in studying small-molecule interactions with proteins in a membrane environment. The technology was used in combination with supported lipid monolayers and vesicle-capture sensor chips to elucidate ligand-binding to different membrane receptors. Gordon Tollin (University of Arizona, AZ, USA) described plasmon-wave guide resonance, which differs from the Biacore SPR because it generates resonance spectra for both parallel and perpendicular planes allowing for better resolution and sensitivity of detection. Additionally, the configuration of the membrane-bound protein in this system is in a more native state and enables analysis of structural events, such as alterations in membrane thickness and molecular orientation that occur on ligand binding. Holger Ottleben presented work using Graffinity's (Heidelberg, Germany) screening platform, which is used to determine label-free affinity binding profiles of GPCRs using massively parallel SPR. Graffinity has teamed up with M-Phasys GmbH (Tübingen, Germany) which has a system to express GPCRs in bacteria with a high degree of active folding. The combination of these two technologies could prove to be powerful in screening GPCRs and other membrane-bound proteins.

Advances in detection technologies

The highlights from this session included the development and testing of two novel HTS instruments for direct electrophysiological measurements of ion channels. Kirk Schroeder (Essen Instruments, Ann Arbor, MI, USA) and Jonathan Trumbull (Abbott Laboratories) both described separate systems that have been developed to measure signals from ion-channel assays in a high-throughput mode.

High throughput ADME/tox

Although hits generated from HTS are increasing exponentially, the process of turning these hits into leads worthy of developing is still a major bottleneck in the process of discovering new drugs. Early and high-throughput methods to determine the metabolic, pharmacokinetic and toxicological properties of hits generated from screening could improve the odds of finding new chemical entities.

Stan Young (GlaxoSmithKline, Raleigh, NC, USA) described a recursive partitioning algorithm for predicting mutagenicity based on his analysis of an Ames mutagenicity dataset. He stressed the importance of carefully defining descriptors and using large and random datasets when building recursive patterning methods. Chris Lipinski (Pfizer Global Research and Development, Groton, CT, USA) dealt with what is important in the early stages of the lead discovery process from an ADME/Tox point of view, defining the most important parameters for oral activity as solubility, permeability and potency. For lead development it is better to start with a lead that has good solubility and permeability properties. Although formulation can aid solubility, it can not help permeability. He also discussed his 'Rule of 5' and correlated polar surface area with permeability. These rules are being followed by Pfizer, not only in the design of new compounds, but also in consideration when preparing screening libraries.

Jeff Sarver from the University of Toledo (Toledo, OH, USA) presented a high-throughput assay for the identification of P-glycoprotein (Pgp) substrates, and Charles Crespi from Gentest Corporation (Woburn, MA, USA) provided an overview of how to design and interpret competitive CYP assays.

The conference ended with a series of awards lectures and an automation session and, finally, a lively and witty panel discussion on the merits of screening, or not screening, large library sets, led, on opposing sides, by Richard Harrison (Dupont Pharmaceuticals, Wilmington, DE, USA) and Carol Anne Homon (Boehringer Ingelheim, Ridgefield, CT, USA).

In summary, the conference was very informative and covered all the cutting edge technologies and advancements in the high throughput arena. The meeting covered not only the lead generation aspect but also the lead optimization and hit-to-lead conversion hurdles. Therefore, the role of ADME applications for converting a hit to a viable lead recieved considerable attention. Similarly, the importance of informatics on collecting, managing and analyzing the large amount of data from HTS was emphasized very well in the conference.

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