me that good academic positions are not vacant for the taking. There are benefits to the academic–industrial relationship to be sure, but its popularity among job and fund seekers, and emulation by non-industrial funding bodies, is not evidence of its success. In a world with no alternatives, I concede Kozlowski's point that industrial relations do not disproportionately impact academic research. However, I fear for the advancement of science in such a world.

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Beyond uHTS: ridiculously HTS?

Newcomers to the world of HTS attending the back-to-back High-Throughput Screening/Miniaturization Technologies conferences at Monterey, CA, USA (28 February–3 March 2000) could have been forgiven for thinking that the industry was struggling a little to get to grips with where its priorities should lie. While some speakers were presenting exciting glimpses of technological developments far beyond the 1536-well plate with ever greater potential throughputs, many others were starting to acknowledge that primary screening is no longer the bottleneck in the pursuit to discover novel lead candidate molecules with therapeutic potential. What has emerged are bottlenecks both upstream, notably assimilating target information emerging from the Human Genome Project into screenable assays, and downstream, coping with the 'rich seam' of hits that ultra-HTS (i.e. that performed in 1536-well plates) is uncovering.

Coming up with the goods

A solution to the hit characterization perspective was offered by Bill Janzen (Sphinx Pharmaceuticals, Research Triangle Park, NC, USA) in his keynote speech, who reminded the audience just how far the HTS industry had come in a relatively short time (Table 1). Before the audience could become too self-congratulatory, he reminded everyone of the key obvious limitation to HTS: it usually identifies only hits rather than leads. He suggested the following definitions:

- Hit – A molecule with confirmed activity from the primary (HTS) assay, with a good profile in secondary assays and with a confirmed structure.
- Lead – A hit series for which the structure–activity relationship (SAR) is shown and activity demonstrated both *in vitro* and *in vivo*.

Janzen offered Sphinx's solution to the dilemma of how to prioritize upwards of thousands of active molecules now being produced from uHTS. He proposed that the solution lies in close co-operation between compound library managers, HTS biologists and discovery chemists under the umbrella 'hits to leads'. Key features are rapid delivery of 'cherry-picked' active compounds for batch potency/selectivity testing and fast cyclical library synthesis based on screen results to see if SAR exists. A key requirement is having access to powerful data analysis software tools to assimilate the vast quantities of data being generated. For Sphinx, results so far are very encouraging, with two-thirds of the projects having yielded leads.

Exploitation of virtual screening

Building on the theme of possibilities downstream of HTS, Andy Good (Bristol-Myers Squibb, Wallingford, CT, USA) contended that virtual screening techniques and HTS have much to offer each other. He claimed that the industry had been slow to exploit fully the extensive quantities of HTS data it had now accumulated. The prerequisite is for the generation of high-quality screen data through maintenance of compound integrity, rigorous compound handling and testing of samples in duplicate to improve reproducibility, which becomes economically more viable

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Table 1. The Janzen interpretation on the evolution of HTS in the 1990s

Year	Throughput	[a]Target lifecycle	Focus/features
1992	1000s week^{-1}	Years	Techniques
1994	10,000s week^{-1}	<1 year	Technology (e.g. FLIPR introduced) 384-well plate – a curiosity Stylli vision of uHTS
1996	1000s day^{-1}	Months	Biology 384-well plate – proof of application uHTS – how to do it?
1998	10,000s day^{-1}	Weeks/days	Genomic targets – reality 384-well plate in broad use 1536-well plate – proof-of-principle
Today	⩽100,000 day^{-1}	Weeks/days	100s/1000s hits-per-target identified uHTS a reality

[a] Length of time a particular target is used for screening before moving onto another target.
Abbreviation: FLIPR, Fluorescence Imaging Plate Reader.

using uHTS. Good suggested a hybrid three-dimensional (3-D) pharmacophore fingerprint search approach. This combines the speed of established 2-D methods such as Daylight and Isis and the versatility of existing 3-D approaches based on target structural information or multiple chemotypes. This makes it possible to fingerprint novel genomic targets with limited structural information or where only one chemotype lead exists.

Dmitrii Rassokhin (3-Dimensional Pharmaceuticals, Exton, PA, USA) introduced a new method to screen virtual combinatorial chemical libraries from more pharmacologically relevant drug-like structures. This is based on multi-objective optimization (diversity, MW and logP) by applying the Kolmogorov–Smirnov equation to enforce given criteria, so overcoming problems of single-parameter selection where the other parameters are compromised (e.g. lowered MW or raised logP). In this way, key molecular drug-like properties can be built into virtual combinatorial libraries. Increasingly, it appears that the battle to identify novel lead candidate molecules will be 'fought' at the computer.

Early ADME considerations

Another trend within the lead discovery value chain is ever-earlier implementation of assays to examine the ADME/tox properties of hits. To emphasize this need, Charles Crespi (GENTEST Corporation, Woburn, MA, USA) reminded delegates that one-third of drug failures are caused by inappropriate ADME properties, such as poor bioavailability or long half-lives. He cited as an example the heart drug Posicor (Roche, Basel, Switzerland), which had to be withdrawn because of adverse drug–drug interactions. He advocated concentrating on screening using five of the liver metabolizing cytochrome P450 enzyme isoforms – 3A4, 2D6, 2C19, 2C9 and 1A2. Chin-chung Lin (Schering-Plough, Kenilworth, NJ, USA) informed attendees that they use 3A4 and 2D6 isoenzymes first and only use 2C9 and 2C19 isoforms later for selected compounds of further interest. He said he saw a need for increased throughputs for drug metabolism and pharmacokinetic assays and felt 'uncomfortable being at the rate-limiting step'. Crespi warned delegates to consider using multiple cytochrome P450 substrates, particularly for 3A4, which has a large active site capable of binding several molecules simultaneously, as compounds can have dichotomous effects. As the Food and Drug Administration recognizes assays using both recombinant enzymes and human liver microsomes, these tests are set for increased prominence in the lead discovery process. The result would appear to be ever-greater stringency in the acceptance criteria to confer lead compound status on molecules but hopefully with fewer failures post lead discovery and a condensed lead optimization process.

1536-well uHTS comes of age

Throughout the week, many speakers discussed their experiences tackling issues such as compound reformatting, liquid handling of nanovolumes and assay detection technologies with 1536-well plates. This prompted Dan Brown (Bristol-Myers Squibb, Wallingford, CT, USA) to claim that in comparison to last year's corresponding conference, this year there had been little justification needed for the use of 1536-well plates and that uHTS 'had truly arrived'.

Several speakers spoke of their experiences with the use of vendor's instrumentation using 1536-well plates. Two products have been established that use cooled charge-coupled device (CCD) cameras and telecentric lenses to simultaneously capture light signals across a 1536-well plate in a matter of minutes – CLIPR (Chemiluminescence Imaging Plate Reader) from Molecular Devices (Sunnyvale, CA, USA) and the LEADseeker from Amersham Pharmacia Biotech (Amersham, Buckinghamshire, UK). As such, both devices have enabled scintillation proximity assay (SPA)-based assays (unquestionably one of the most established assay formats both in HTS and in therapeutic groups) to be miniaturized down to the 1536-well plate level for the first time. Brown outlined his group's experiences of testing [^{3}H]-YSi beads using CLIPR, noting

that they saw lower signal–background ratios but improved coefficients of variance (CVs) compared with photomultiplier tube-based readers, being an acceptable compromise for the much greater throughput. Using the LEADseeker, Suzanne Green (Glaxo-Wellcome, Stevenage, Hertfordshire, UK) said that her group demonstrated similar pharmacology of standard inhibitors for several assays (including a viral protease and a purinoceptor binding assay) to that obtained using lower-density 96-well plates. The main technical issues they were addressing were how to cope with plate phosphorescence, bead settling prior to dispensing into 1536-well plates, and the stand-alone nature of both CLIPR and LEADseeker.

At least three companies have developed new detection technologies to enable homogenous fluorescence assays to be established. Christine Pernelle (Aventis, Paris, France) discussed their experiences of using ACUMEN (developed by The Technology Partnership, Melbourne, UK), which is a device with a 488 nm laser scanning instrument with four emission channels capable of scanning areas of 400 mm^2 at speeds of 2–5 ms^{-1}. The system has been established for various assay formats including on-bead tyrosine kinase and reporter assays. Sheri Miraglia (PE BioSystems, Gaithersburg, MD, USA) discussed their Fluorometric Microvolume Assay Technology (FMAT) that uses a 633 nm He–Ne laser and two photomultiplier tubes set at 650 nm and 685–720 nm. They have established a new assay principle called Fluorescent-Linked Immunosorbent Assay (FLISA), in which antibody-coated beads of two different sizes are used together for in-well multiplex detection, with the described example being simultaneous cell surface intracellular adhesion molecule 1 (ICAM-1) detection and interleukin 8 (IL-8) secretion. Tom Mander (Evotec) updated delegates on the progress of the Fluorescence Correlation Spectroscopy (FCS)+*plus* platform for both solution-based and particulate (e.g. vesicles and whole cells) assay formats.

Meanwhile, Tina Garyantes (Merck & Co, Rahway, NJ, USA) described a novel, double-sided compound storage plate whereby compounds are stored as 1–2 μl 'spots' on the lower, sealed side in larger wells and 2.5 nl is taken from the smaller wells of the upper side using a 1536-pin tool. Pin-width channels connect the two sides, serving two purposes: to minimize evaporation and to enable a uniform residual volume to be retained on the tip end on withdrawal of the tool.

A future beyond 1536-well plates?

Despite the technical challenges associated with establishing uHTS platforms at the 1536-well plate level, some companies have already embarked on developments far beyond the 1536-well plate. Mike Needels (Affymax Research Institute, Palo Alto, CA, USA) opened the conference by anticipating the need to increase screen throughputs to 10–100 million compounds-per-target to increase hit success rates. He talked of developing a 20,000 well plate with 25 nl-per-well volumes, which would conceivably enable the screen capacity to reach 1.5 million compounds per week.

Later in the week, Jim Kofron (Abbott Laboratories, Abbott Park, IL, USA) presented an update on their Continuous-Format (CF)-HTS platform, an 8,640 well-less plate where 37 nl of compounds (150–250 pmol) are spotted onto a sheet and allowed to dry before adding assay reagents impregnated into agarose gel on top. Up to 12 assays have successfully been run of varying formats, including cell-based (e.g. reporter, cytotoxicity and Ca^{2+} uptake), SPA and enzymatic assays. Kofron acknowledged that a 'huge group of people had worked on this' and said that six man-years were required for the software development alone.

Concluding remarks

Although the industry outsider's take-home message from the conference could have been that the HTS industry has made tremendous technology strides over the past 5–10 years, it has perhaps been a bit over-obsessed with numbers and throughputs at the expense of being true hit discoverers. Refreshingly, people are now beginning to address new bottlenecks in other aspects of the lead discovery process in a more rigorous way, including the hit profiling and target validation processes. David Nelson (Aurora Biosciences, San Diego, CA, USA), while talking about their GenomeScreen™ efforts that focus on the introduction of their β-lactamase reporter gene randomly into cells, came up with my quote of the week suggesting that screening beyond uHTS was akin to 'ridiculously HTS'. For some, this might be an uncomfortable message, but if we are to be seen as successful in years to come, it is important we focus on using the powerful technologies now available wisely to help generate the medicines of tomorrow to justify the not inconsiderable capital investments our companies have made in HTS.

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