Fine-tuning the technology strategies for lead finding

HTS has become the workhorse of pharmaceutical and biotechnology companies' drug discovery efforts, with expanding responsibilities and increasing pressures to screen more targets with better compound libraries to find high-quality leads.

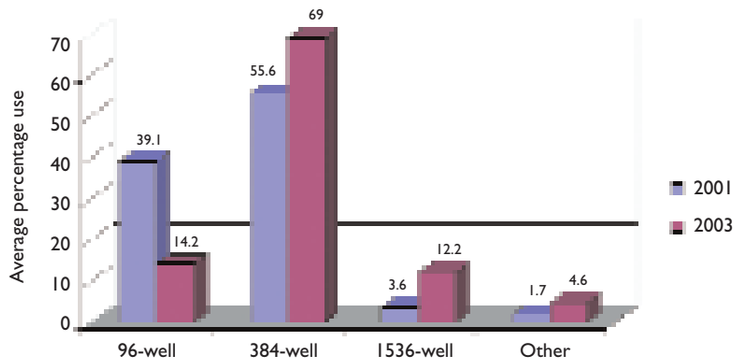
By Sandra Fox, Dr Shauna Farr-Jones, Dr Lynne Sopchak and Dr Helen Wang

HTS groups have taken on new responsibilities and new roles. Almost all HTS laboratories now conduct secondary screens, and some conduct *in vitro* ADME/Tox screens. There is a concerted effort to improve the quality of compound libraries, increase throughput to accommodate more compounds and more targets, and find more leads that become clinical candidates. Based on the new HTS study 'High-Throughput Screening 2002: New Strategies and Technologies', innovative organisational structures are emerging, including a matrix structure whereby one team of experts, including a representative from the HTS group, sees one project all the way through to clinical trials. Some HTS groups are becoming more involved throughout the drug discovery process, from target validation to lead optimisation and *in vitro* ADME/Tox studies. For some, high information content screening is becoming part of the screening philosophy. Fifty-one HTS directors were interviewed for the 2002 HTS study.

Miniaturisation strategies

The HTS groups are pressured to find leads quickly, causing the average number of targets screened and the average number of compounds screened per target to increase year to year. HTS directors of the larger industrialised laboratories now predict they will be screening one million compounds per target on average by 2003 rather than by 2005 as had been predicted earlier. To accommodate these increasing throughputs with limited budgets, HTS directors are continuing to implement miniaturisation strategies.

Figure 1: Average percentage use of microplate formats, 2001-2003



Note: Other includes 3456-well microplates, microfluidic chips, microarrays, electrophoretic tools, mARCs and an agar-based embedding technology

Source: HighTech Business Decisions

Most participants in the current HTS study are using 384-well microplates in their HTS operations. To decrease costs and increase throughput, many HTS laboratory directors are planning to use higher-density plates or microfluidics for both cell-based and biochemical assays. The expected changes in use of various microplate formats are shown in **Figure 1**.

The HTS directors in the 2000 study 'High-Throughput Screening 2000: New Trends and Directions', predicted 1536-well plates would represent 12.2% of their screens on average by 2002, which apparently has been pushed out to 2003 based on the new study's results. HTS directors primarily cite problems with liquid handling and detection sensitivity as the causes for the delay in moving to 1536-well microplates. In addition, transferring compounds from storage plates to assay plates with higher density formats has posed problems for those changing to 1536 formats.

Now, however, several suppliers offer solutions to the liquid handling problems associated with small volumes and 1536-well microplate formats. Cartesian Technologies, acquired by Genomic Solutions (Ann Arbor, MI) in December 2000, recently introduced the Hummingbird™ System. This is a tabletop dispenser that uses capillary action for non-contact, parallel liquid transfers of nanolitre volumes. Barbara McIntosh, HTS global product manager for Genomic Solutions says: "In less than 30 seconds, for example, a plate can be replicated from storage to the assay plate, 384 wells to 384 wells. The system can also handle transfers from 96-well plates to 384 formats, and even to 1536." Because pressure from the capillary action does the work, there are no pumps involved, and colour-coded cassettes deliver fixed nanolitre volumes.

The Cartesian Hummingbird System is designed to work with the company's synQUAD™ dispensing system, which adds reagents to the assay plates. McIntosh explains: "There are no wash steps required after dispensing, and both synQUAD and Hummingbird can be integrated with Zymark's Twister™ microplate handlers."

In September, Amersham Biosciences (Piscataway, NJ) will introduce a LEADseeker™ multi-modality imaging system to address the needs for rapid quantitation of assays in a range of formats, including 1536-well microplates. The new LEADseeker will extend the use of LEADseeker from radiometric, luminescent and FRET modes to include fluorescence polarisation and time-resolved fluorescence. Penny Owen,

The Cartesian Hummingbird System

Corning's 384-well low-volume microplate

Marketing Director, High-Throughput Screening, says: "Use of imaging rather than PMTs improves the precision of detection, and therefore the robustness of the assay, offering greater confidence in the identified hits. This is especially important for assays where the signal window is small because a less precise system would not be sufficiently robust."

The LEADseeker has a delivery mechanism that can load or unload a plate in less then 10 seconds, and can be accessed by a range of plate-handling systems. In addition, Amersham offers a range of matched reagents: beads for radiometric proximity assays, CyDye fluors and generic TRF reagents. These reagents are red-shifted to match the CCD and maximise sensitivity. Owen says: "Red-shifting the reagents has the advantage of reducing the main causes of false positives: colour quench from compound libraries and background fluorescence. For fluorescence assays, Cy3B offers a large signal window, improving assay precision and increasing confidence in the quality of identified hits. A new bead type for radiometric proximity assays that was introduced this year is an arginine-binding bead for NO synthase assays."

Benefits of low-volume 384-well microplates

Those HTS directors interested in low volumes and higher speeds are investigating 1536-well plates and microfluidics, while those primarily focused on reducing the reagent volumes are using low-volume 384-well plates. In the new HTS study, the number of HTS directors pursuing a 1536-well plate strategy is the same as the number pursuing a low-volume 384-well microplate strategy. Several HTS directors have found that low-volume 384-well plates provide easy access to the advantages of low volumes typical of the higher density formats.

Corning Life Sciences (Acton, MA), recently introduced a 384-well low-volume microplate with a non-binding surface to meet the needs for

screening at low volumes. These plates have well volumes of 35 microlitres and working volumes of 1 to 20 microlitres. In addition to the low volumes, the new plate configuration has some added advantages. Jill Veilleux, Worldwide Marketing Manager, says: "One of the major problems that assay development scientists have been plagued with is the formation of bubbles, which can cause high CVs and can also be misconstrued as 'hits' (false positives) when using common CCD field imagers for detection. The 384-well low-volume well shape and non-binding surface eliminates the formation of these bubbles. The non-binding surface also enables the miniaturisation of homogeneous assays, by allowing customers to reduce the concentrations. These plates provide not only reduced reagent consumption and lower assay costs, but increased signal-to-noise ratios."

More cell-based assays in HTS mode

The HTS directors are almost evenly split between believing that success for HTS in finding commercial therapeutics is just around the corner versus those who believe changes need to be made to HTS to make it more effective and more successful. These suggested changes include the use of better compound libraries and conducting secondary and *in vitro* ADME/Tox screening to better qualify leads. In addition, using better validated targets and combining HTS with molecular modelling, virtual screening and data analysis tools are likely to increase success rates.

Almost all of the HTS directors interviewed for the 2002 HTS study are conducting both primary and secondary screening to find high-quality leads. One-fourth are conducting some *in vitro* ADME/Tox screening or are planning to do so in the near future. As such, the growing focus is on cell-based assays.

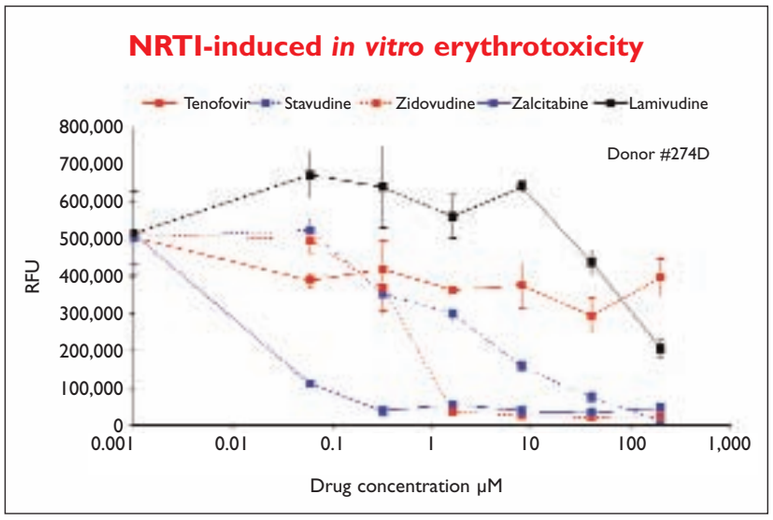
Tecan Group (Maennedorf, Switzerland) has a new instrument, the Genesis® Freedom™ Automated Workstation, which is a liquid handling system for *in vitro* biochemical assays and cell-based assays. It has been designed to easily integrate into the HTS line with sample prep instruments for cell-based assays, such as flow cytometers and centrifuges. Johanna Neumayer, Marketing, Assay Development, says: "For the past two years, Tecan has focused on *in vitro* ADME/Tox screening so cell-based assays such as cell permeability can be done on workstations. Our detector, SPECTRAFluor Plus, can be used for cell proliferation, cell migration, apoptosis and other relevant specialised cell-based assays. It can read cells from either the top or the bottom of the plate; cells that are at the bottom of the well or in suspension can be read."

The Genesis® Freedom™ automated workstation from Tecan Group

Tecan's ULTRA multi-functional detection system can also be used for cell-based assays, adapting to several assay formats including 1536-well microplates. In addition, to solve high-density liquid handling problems, Tecan has introduced a new multi-channel pipetting system, Te-MO, that works with the company's Genesis system for 1536-well microplate dispensing."

Year to year, HTS directors continue to predict an increasing use of cell-based assays as their responsibilities expand. In response to the need for more cell-based assays, several suppliers are providing the cells, assay platforms, assay development services and imaging systems.

Cambrex, Inc (East Rutherford, NJ) provides primary human cells and cell-based assays to the research community through its BioWhittaker, Clonetics and Poietics brands. Dale Greenwalt, PhD, Director of Bioassay Development says: "We



CELISA-based high-throughput *in vitro* safety evaluation
CELISA assays were used to screen nucleoside reverse transcriptase inhibitors for erythrotoxicity in cultures of human bone marrow-derived progenitors

have three main product categories: primary human cells and media, high-throughput cytotoxicity and cell proliferation assay reagents, and assay services and custom assay development based on our CELISA™ platform technology. Our primary human cells are used for both HTS and high information content screening. There are several advantages to using normal human primary cells for screening instead of cell lines or animal cells. For example, species-specific effects can be avoided."

Cambrex has a homogeneous luminescent cell proliferation assay, ViaLight™, that requires the addition of a single reagent; the plate can then be read immediately in HTS mode. The company recently introduced a new HTS assay, ToxiLight™, also based on the bioluminescent luciferase reaction, for cell toxicity. Greenwalt says: "The advantage of ToxiLight is that the supernatant is used in the assay and the cells can be left in the culture plate for use in a second assay. The assay is robotics compatible and non-destructive."

Cambrex is turning old-fashioned assays, which are typically low-throughput, expensive and labour intensive, into high-throughput assays. For example, whereas technicians typically count cells or colonies in traditional assays of hematopoietic progenitor cell differentiation, Cambrex has an array of new HTS cell differentiation assays that can be measured in 96-well plates.

Greenwalt explains: "CELISA™ is a 96-well filter plate assay that can quantify the differentiation of hematopoietic progenitors into mature cells of three different lineages (myeloid, erythroid and megakaryocytic). You assay the degree of differentiation by measuring the expression of lineage-specific cell surface markers with a fluorescent probe. These assays can be done in a matter of minutes automatically instead of having a technician count colonies all day. You get much higher throughput for both drug discovery and safety evaluation applications." CELISA is currently offered as a service, and kits are in development. The CELISA-based bioassay and assay development services are available for use with a number of cells including hematopoietic progenitors, preadipocytes and osteoclast precursors.

Universal Imaging Corporation (UIC, Downingtown, PA), is focused on imaging technology for high content screening applications. UIC's Discovery-1™ is a completely automated system, including robotics support, that allows acquisition of image data at 4-60 times magnification from individual wells in all microplate formats.

"We've leveraged off our 18 years' experience in bio-imaging to produce a system that can operate in a turnkey fashion for common assays such as apoptosis, neurite outgrowth, cell counting, morphology and compartmental redistribution. The system also allows users the flexibility to easily develop their own assays," says Dr Jeff Stuckey, CEO. "Discovery-1 provides an increasingly important component of drug discovery programmes as functional genomics is driving a need for assays that measure parameters at the cellular and sub-cellular level."

Addressing the bottlenecks

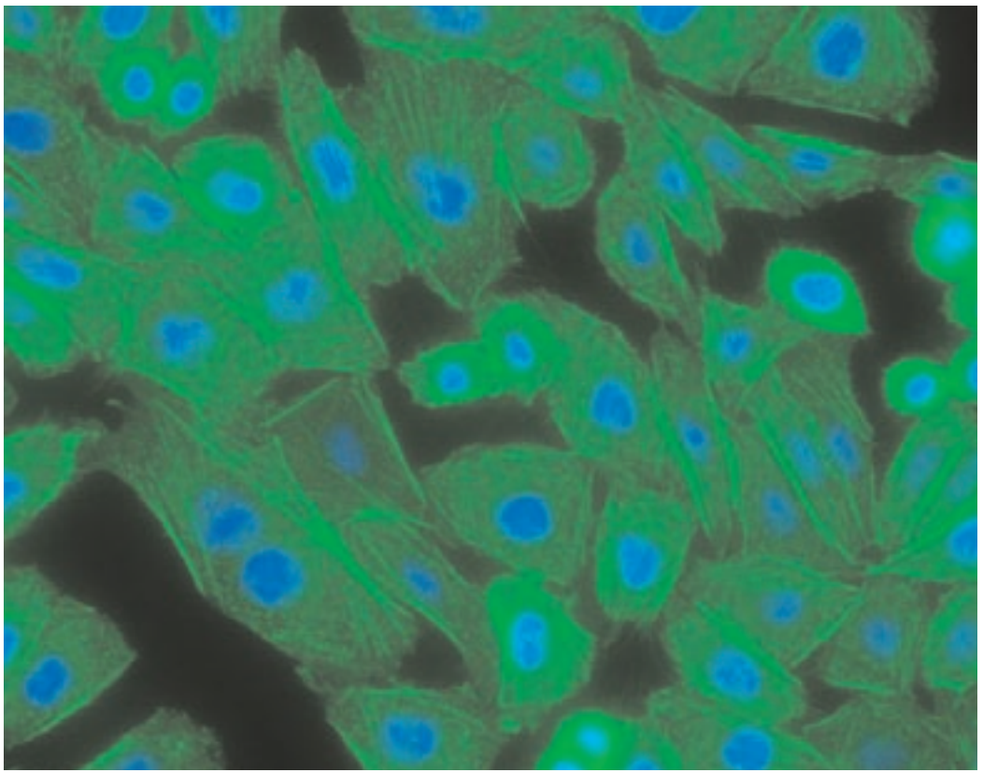
Assay development continues to be a bottleneck for HTS. With increasing numbers of targets and new target types, it is difficult to streamline the assay development process and still produce a robust miniaturised assay with customised reagents. Reagent and protein production often cause delays in the implementation of the screen.

Significant input and co-ordination from diverse functional groups are required to produce validated assays for HTS. For example, validated assays require protein expression and purification to produce the soluble, active drug target protein. In addition, chemistry expertise is needed to produce fluorescent detection reagents or derivatives of the drug target protein. Then the assay must be developed, validated and optimised for HTS. This can be especially difficult to co-ordinate if resources and functions are spread out among global sites and the company is dealing with numerous targets.

Not surprisingly, the most often mentioned bottleneck in HTS overall is assay development, as reported by 51 HTS directors in the new 2002 study.

To solve some of the assay development problems, PanVera Corporation (Madison, WI) provides homogeneous fluorescent assay platforms for important drug target families. Randy Bolger, PhD, Director of Business Development, says: "An industrial mindset and process is required to deliver an increased number of assays in a cost-effective way. At PanVera we are expanding a panel of off-the-shelf assays around target families, such as the kinases, so that primary screens can be performed quickly and efficiently. The identified hits can then be moved directly into parallel, counter-screening using the same assay and format. This approach reduces the need to retool the HTS platform for each new screen."

PanVera's expertise has expanded beyond fluorescence polarisation (FP) to include FRET and proprietary TR-FRET methods. For example, an FP-based kinase assay panel has been developed



Cytoskeletal Morphology Apoptosis Assay
TRVb-1 cells stained with Alexa 546 Phalloidin (535/610) and Hoechst (360/480). Image acquired with Universal Imaging Corporation's Discovery-1 Cell Based Screening System, 2002

using peptide substrates that provide maximum kinase coverage. In addition, FRET-based kinase assays using Aurora's PhosphoryLight™ technology will provide greater flexibility in the design of kinase assays around specific substrates. FP and TR-FRET are being used in the development of a suite of assays around nuclear receptors to probe both the hormone and co-regulator binding sites. PanVera has also introduced cytochrome P450 (CYP450) turnover assays; combining highly active recombinant P450's (BACULOSOMES®) and the Aurora Biosciences Vivid™ fluorogenic P450 substrates.

Alex Vodenlich, Vice-President of Contract Services says: "We are combining our expertise in protein expression and production, fluorescent chemistry and HTS homogeneous assay development to manufacture and deliver a reliable supply of robust assay reagents; available in the form of off-the-shelf kits or through confidential contract services. Assays are lot controlled, custom dispensed and packaged, and delivered globally according to a client's specifications. Decreasing the time of screen implementation and follow-up without sacrificing data integrity is what it's all about."

The build-up of equipment and investments in HTS has resulted in increased capabilities at individual HTS laboratories, which now have greater capacity than ever before. For some HTS laboratories, however, having enough validated assays to run in this new industrialised environment has become a significant bottleneck. DDW

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