Increasing the chances of lead discovery

For HTS laboratories worldwide, the mission is to supply therapeutic groups – in the shortest time possible – with high quality hits and leads that will become drug candidates. Mounting pressure to screen more targets against more compounds while providing more information per screen has HTS directors seeking improvements to existing technologies as well as innovative new approaches and tools.

By Sandra Fox, MBA, Dr Helen Wang, Dr Lynne Sopchak and Dr Richard Khoury

The results of a recent survey of 52 HTS directors at pharmaceutical and biotechnology companies indicate that the number of compounds being tested per screen is increasing dramatically. On average, in 2000, 300,000 compounds were screened against each target. By 2005, HTS directors expect the number of compounds tested per screen to be well over one million. Although major pharmaceutical laboratories are currently testing more compounds per screen on average than the smaller pharmaceutical and biotechnology company laboratories, by 2005 both groups expect to be on par with more than one million per screen on average. This is shown in **Figure 1**.

HTS directors are expecting to increase their throughput substantially to accommodate this increase in the number of compounds screened. By 2005, almost half of the HTS directors interviewed expect to be reading more than one million wells per week. One HTS director explained: "We will increase the number of wells read per week in order to get more bang for the buck. Miniaturisation is another reason it will increase, with a 384-well microplate being as easy to screen as a 96-well plate is now. The rate of attrition in HTS is based on the fact that the more targets and compounds you get through, the greater the chance of getting a clinical candidate." Several factors affect the ability to reach these high-throughput levels, however. One HTS director cautioned: "We will increase our throughput in five years and it may double. Who really knows? We'll have to make a special case to buy compounds so it depends on how well the combi-chem people do."

In addition, some HTS directors are planning a different strategy that does not require more compounds and throughput, but does require more focused screening and more information per screen. One HTS director explained: "The number of compounds will stay the same as we'll use more screens with selected focused libraries. We expect on-line control. The first 1,000 compounds will be identified and a certain hit pattern will guide us better with no need to do massive primary screens." Overall, the debate regarding throughput versus information in HTS operations is undecided, with HTS directors almost equally divided between a 'high-throughput' approach, the 'more information' approach, or a combination of the two.

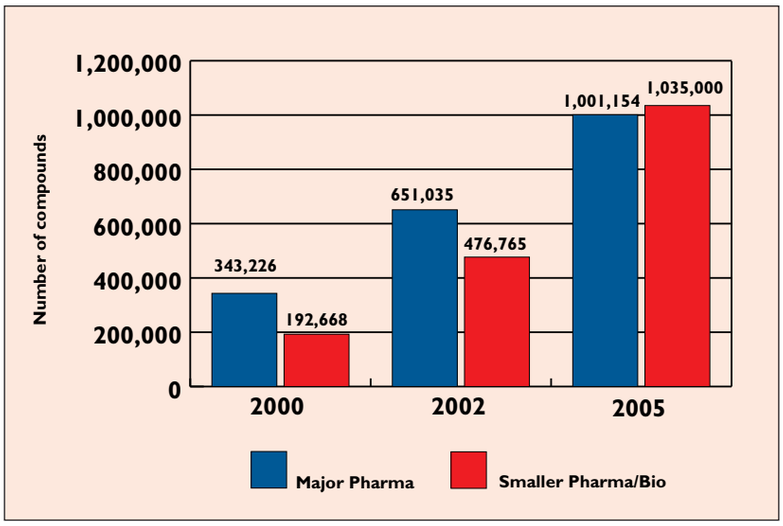


Figure 1
Average number of compounds tested per screen at HTS laboratories. Source: HighTech Business Decisions

The best assays

Another area of debate is the question of assays and which ones provide the best quality leads with the highest probability of reaching clinical trials. In the survey, HTS directors listed numerous assay types as the best ones to use. Those most prevalently mentioned include enzyme assays, cell-based assays, and assays for GPCR targets. Some mentioned detection modes such as fluorescence and luminescence. For the most part, directors acknowledge that no one assay has been identified as more useful for finding leads. Some say that the assay is not the determining factor, but the type of target is. Others say it is simply too early to tell.

The use of cell-based assays in primary screening is a controversial issue as well. On average, the HTS directors participating in the survey expect their use of cell-based assays to increase in the next two years. Some believe cell-based assays are the most relevant assays for finding hits and leads and they are increasing the percentage of cell-based assays in primary screening, while another similar number of HTS directors find cell-based assays difficult to work with and are decreasing their percentage use. However, several directors who are planning to decrease their percentage use of cell-based assays say that even though the percentage may be expected to decrease, the actual number of cell-based assays will still increase owing to the expected growth in overall throughput in primary screening.

Directors were asked to comment on their changes. One strategy is to use more biochemical assays in primary screening while using the cell-based assays as a follow-up assay later in the hits-to-leads process. Some believe you can find hits faster with biochemical assays, but you find leads faster with cell-based assays. Others, who had not used cell-based assays before, are finding they are valuable for quickly finding out if a drug affects a cell line and these directors plan to increase their use of cell-based assays in primary screening.

Industry resources

Several suppliers are developing new products and services to help clients increase the chances for lead discovery. At **Amersham Pharmacia Biotech** (Little Chalfont, UK), Neil D. Cook, PhD, Vice-President of Drug Discovery, says that pharmaceutical companies are seeking improvements in comparative bio- and chemi-informatics and in the hit-to-lead process, more commonly called secondary screening. Because HTS operations are now effective in most pharmaceutical companies, hits are being generated in much shorter timeframes, creating a bottleneck in the secondary evaluation process. Cook explains: "The challenge is to adequately analyse these hits quickly. Failure to do so would eliminate all the advantages gained from developing the efficient HTS process that has been the focus for the last three to five years in many pharmaceutical discovery organisations. One solution is to run so-called 'smart' assays in parallel at relatively high speed, assays that generate much more information. The use of cellular assays is likely to be a major focus in assessing these hits."

By running a number of well-defined assays in parallel for key parameters that indicate beneficial pharmacophoric properties, hits may be selected on more complex criteria. Assays to determine uptake into cells or tissue types, *in vitro* metabolism, and toxicological effects may be performed as well as those assays that test relative potency of the hits and analogues of the chemical family. Cook says: "By utilising expert sample workflow, data systems, and comparative informatics, the hits can be analysed in a much more intelligent way."

Amersham Pharmacia Biotech is currently in the process of developing a multiparameter Cellular Analysis system specifically for the secondary assay process. The first generation of this technology, the LEADseeker™ Cell Analysis System, provides rapid multicolour analysis of live-cell assays in static and kinetic modes. Cook says: "The new system will be developed to cover aspects of the hit-to-lead process with assays, reagents and software capable of performing multiparameter High Content Screens for hit validation and *in vitro* ADME/Tox applications. By utilising advanced sample and data workflow systems, we will link our many technology platforms together into parallel data streams: gene expression data from microarrays, protein expression from 2-D analysis, and cellular physiology data from the Cell Analysis System."

Mark Roskey, PhD, Director of Pharmaceutical Screening, at **Applied Biosystems** (Applera Corp), says: "The top problems for HTS are moving away from mechanical issues of robust robotics and high/ultra high-throughput screening capabilities to more 'assay' or biological issues. The overall problem is to develop assays and assay technologies that accurately represent a relevant biological phenomenon, yet are robust enough to run robotically without generating large numbers of false positives." Roskey observes that HTS assays need to become capable of generating additional information on biological relevance and the toxicology of a test compound. In some cases, direct binding assays and reporter gene type assays are being blended to create new assay formats that are more cell-based yet specific for the interaction of interest.

Applied Biosystems is developing specific assay

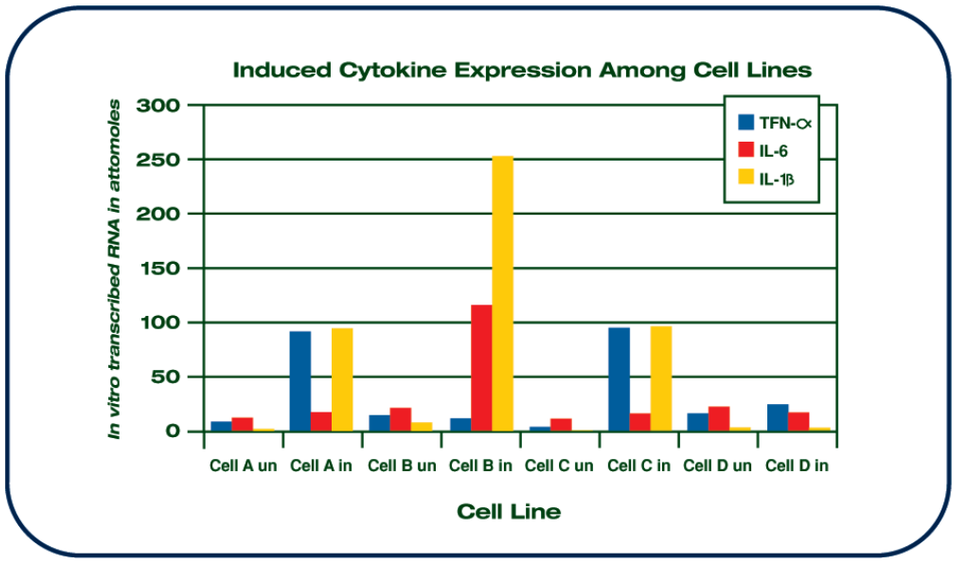
The ArrayScan® II System from Cellomics, Inc

technologies to achieve a higher overall quality of leads from HTS programmes. Roskey explains: "We have introduced an ultra-sensitive assay for the measurement of cAMP, which has a significantly reduced false positive hit rate. Additionally, we have developed a protein:protein interaction technology (ICAST™) that measures specific protein interaction directly in mammalian cells in the cellular compartment in which these interactions occur. This provides hits with a higher level of biological relevance. A range of specific biological assays are currently being developed for the FMAT™ platform that are designed to directly mimic biological pathways such as apoptosis and cytokine signalling."

At **BD Biosciences** (Bedford, MA), Blake Perkins, Marketing Manager at Discovery Labware, says he has observed that pharmaceutical and biotech companies are improving the chances of finding successful lead candidates using a three-prong approach: 1 Seeking or developing data mining 'bioinformatics' software that can predict lead compounds, 2 Utilising innovative 'high content' *in vitro* cell-based assays and products that provide functional predictive models before using costly in vivo preclinical testing, and 3 Standardising and simplifying such products to fit their HTS workflow. Perkins explains: "For example, some HTS directors are automating cell culture and handling activities, moving high content cell-based assays to higher density lower volume formats, improving detection strategies to derive more information per determination, and finally, pushing secondary screening tests 'upstream' to accommodate high-throughput demands."

To assist customers with these new strategies, Perkins says: "BD Biosciences has developed and introduced new products that speed drug development and provide valuable information for qualifying candidates and predicting drug behaviour." Some of these products include BD FluoroBlok™ insert products, which provide homogeneous, real-time formats for high-throughput testing of cellular invasion and migration, and the BD Oxygen Biosensor for high-throughput, reversible determination of cellular toxicity and metabolism, also suitable for anti-bacterial and anti-fungal screening. The company also provides HTS laboratories with BD BioCoat™ Caco-2 products for automation-compatible, Caco-2 testing in 24-well and 96-well formats, BD Falcon™ specialised and bar-coded HTS plates, and BD BioCoat custom plate coating.

Paul Kinnon, VP of Sales at **Cellomics®, Inc** (Pittsburgh, PA), has observed that while genomics and proteomics provide significant insight into the correlation of target identity and expressivity with various disease states or compound treatments, they fail to provide functional screening mechanisms. What is needed is an understanding of the spatial and temporal relationships of various chemicals and cellular proteins within the biologically relevant context of the living cell. Kinnon explains: "We have pioneered a new screening methodology called High Content Screening (HCS) that generates high biological content data regarding the effects of potential new drugs on multiple cellular targets on a cell-by-cell basis and in the same cell population. With fluorescent reagents and



Chromagen, Inc's STATE™ assay technology

Differential expression of multiple cytokine genes measured simultaneously in four different cell lines under normal and induced conditions. *The graph illustrates the application of* STATE" *technology in a 96-well array format, to the simultaneous analysis of three different cytokine genes, TNF-α IL-1β and IL-6, measured in four different cell lines under non-induced (unZ) and induced (in) conditions. The results are quantitative, with the capability of covering six orders of magnitude.* STATE" *assays allow the measurement of individual gene expression and the inter-relationship between multiple gene targets.*

cell lines, advanced imaging instrumentation, and both informatics and bioinformatics tools to transform cell data into knowledge, more efficient validation of cellular drug targets as well as more effective lead optimisation can be achieved. The beauty of HCS is that it simultaneously analyses multiple interacting or independent targets in intact cells. Through this approach, drug screening is based upon target activity, location and kinesis, as well as interacting cellular components and pathways, morphological events and environmental factors that combine to elicit a biologically relevant whole cell response."

Cellomics' ArrayScan® II System, provides a platform for multi-channel fluorescence-based screening of cells growing on standard high-density microplates. Kinnon says: "The cell image processing software we use to quantify changes in fluorescently labelled cellular components are optimised for each assay type to extract consistent results from acquired cell images, optimised signal-to-noise ratios, and minimised co-efficients of variation." In addition, Cellomics also offers fluorescence-based HCS reagent kits as its HitKit™ series. Although optimised for automated analysis on the ArrayScan II System, whole cells prepared from the kits may be analysed by standard fluorescence microscopy platforms. A total of 16 kits are available with key targets in basic signalling, receptor activation, apoptosis, cytotoxicity, morphology and motility.

In addition, Cellomics has just released its new bioinformatics tool, Cellomics CellSpace™ Knowledge Miner, a knowledge mining system that automatically detects, analyses and reports the logical relationships between molecules discussed in the research literature of molecular and cell biology. Kinnon says: "CellSpace™ is a Java-based Web application, providing a textual and graphical interface whereby users can explore the 'knowledge space' of molecular cell biology: a vast collection of scientific literature and current information contained within the proprietary Cellomics database."

David Blair, Marketing Manager at **Chromagen, Inc** (San Diego, CA), says: "We have observed increased testing of highly targeted – rather than random – chemical libraries and a greater number of targets being tested against these libraries. Now, more than ever, robust and rapid assays that are automatable, homogeneous, and miniaturised for

savings on reagents and samples are in demand at these HTS laboratories."

Chromagen provides the STATE™ assays (Simultaneous Transcriptome Analysis of Target Expression), which quantitatively compare the expression of many different genes and are used to select the genes that offer the greatest potential as therapeutic targets. Blair explains: "By identifying the genes that exhibit the most significant changes in expression at each stage of a disease, they provide a bridging technology from microarrays to target gene selection, validation and optimisation."

The first STATE™ assays were developed as gene expression tests, using a fluorescent detection system and DNA probes for cytokine arrays. The second-generation of STATE™ assays will evaluate the genes that encode for drug metabolising enzymes (cytochrome P450s). Blair says: "STATE™ technology utilises simultaneous dual mRNA assays and StarBright® fluorophores in a medium density microwell array format to generate expression profiles for families of pharmacologically related genes in cell lysates. These assays are sensitive with wide dynamic ranges that enable the quantitative evaluation of complex gene expression profiles under a range of induction protocols, normal and disease conditions, and drug screening regimens."

Jill Veilleux, Business Development Manager at **Corning Corp** (Acton, MA), finds that many of the HTS directors are seeking ways to more effectively and more efficiently use existing tools to increase their success at lead finding. Veilleux says: "Although the basic technologies already exist there is still a long way to go in understanding how to apply these technologies in a way that will help accelerate the drug discovery process. For HTS, this means a greater focus on predictive capabilities and improving its ability to integrate and leverage information provided by the other areas of drug discovery."

With the drive towards miniaturisation, HTS directors are becoming increasingly aware of the impact surface to volume ratio has on their assays. To address this, Veilleux says: "Our proprietary Non Binding Surface technology, NBS™, allows our customers to effectively miniaturise their homogeneous assays. In an effort to address the problem

of non-specific binding, we have applied NBS™ to our entire line of high-density microplates including the new 384-well Low-Volume plates and the new line of 1536-well plates. In addition, Corning offers a wide variety of capabilities and expertise, including basic materials science, knowledge of photonics and mass manufacturing techniques that allow us to continue to introduce important new products into the Life Sciences marketplace."

At **Promega Corp** (Madison, WI), Walter Brandes, Business Unit Manger, HTS, says: "Although the holy grail in lead identification and qualification is still higher throughput, the means of achieving improved throughput has evolved to using assays with a higher information content." Cell-based assays, such as reporter gene assays, allow the testing of 'systems' for their response to compounds. Brandes notes that whether the target is a kinase or a GPCR, the testing of a potential lead compound against a pathway in a cell – as opposed to an *in vitro* test of a single event – best meets the screener's goal for all compounds of 'failing fast'. "The development of cell-based assay systems that allow rapid, cost-effective, one-step, homogeneous assays in high density formats is high on the lead discovery list," says Brandes.

Bioluminesence has been around for a long time and, even though appearing to be in the shadow of fluorescence, continues to offer effective solutions to the HTS assay developer and screener. Brandes explains: "In the last several years, Promega's Firefly and Renilla-based luciferase products have been configured to allow the efficient and effective processing of large numbers of cell-based screens using one-step, homogeneous assays in high-density formats. Long half-life reagents that allow readings as much as five hours after reagent addition enable high volume, fully automated batch mode testing on simple robots. High substrate content reagents provide very bright, moderate half-life products, ideal for detecting weak promoters; and dual reporter systems provide for the internal controls so important in 384-well microplate screens." To address the evolving trend toward prescreening libraries for compound toxicity in the HTS environment, Promega also offers new products using luciferin/luciferase technology for a simple and sensitive cytotoxicity screen.

Bottlenecks are occurring later, after primary screening and during data analysis, secondary screening, pre-clinical screening in animal models, and ADME/Tox testing. These will still require significant research efforts to overcome. Undoubtedly, the new products in the marketplace, including those mentioned here, are solving problems for HTS and these downstream processes. They support a journey from hit to lead that has more robust assay technology and detection, and qualitatively, one that is more representative of the disease situation with emphasis on the use of imagers and high-throughput cell-based assays. Furthermore, predictive software, computational chemistry and more focused screening offer ways to minimise costs and time so that lead discovery can proceed faster in a more effective and less expensive way. Together, the new strategies and new products are increasing the chances of lead discovery. DDW

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