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## High-Throughput Screening in Industry

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### CONTENTS

INTRODUCTION  
HIGH-THROUGHPUT ASSAY METHODOLOGIES  
AUTOMATION FOR HIGH-THROUGHPUT SCREENING  
SCREEN MINIATURIZATION  
COMPOUND LIBRARY MANAGEMENT  
HIGH-THROUGHPUT ADME-TOX ASSAYS  
DATA MANAGEMENT FOR HIGH-THROUGHPUT SCREENING  
CONCLUSIONS

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## INTRODUCTION

### *1.1. Anticancer Drug Discovery Today*

Drug discovery operations have been transformed over the past 20 yr by a series of technological innovations and scientific advances that the pharmaceutical industry has been quick to exploit. The trend has been to embrace automation and high-throughput techniques and to integrate research functions into a consolidated framework for drug discovery.

Truly automated high-throughput screening (HTS) dates from the late 1980s and was one of the earliest of the current new wave of technological innovations to be adopted for drug discovery. HTS involves testing collections (“libraries”) of hundreds of thousands of natural products or synthetic compounds against a biological target using a quantitative bioassay. Its purpose is to identify screening “hits” that modulate the activity of the biological target and that form the starting point for a collaborative discovery effort between medicinal chemists and biologists. In essence, HTS is an effective way of reducing a prohibitively large number of diverse chemical starting points to a few promising structures that can be explored in more depth. Initially, the goal of the collaborative effort is to identify “lead” compounds from among the hits. Ultimately, the goal of drug discov-

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ery is to transform lead compounds into drug candidates for clinical development. The strategic importance of HTS is that it requires no prior knowledge of the types of structures that will modulate the target molecule.

Bioinformatics and functional genomics have expanded the number of biological targets available for drug discovery. Furthermore, high-throughput screens for ADME-Tox (absorption, distribution, metabolism, excretion, and toxicity) have improved the chances of identifying better quality, more drug-like lead compounds. The new approaches to drug discovery, when combined with more traditional research methods, are starting to bear fruit, and we are now witnessing the emergence of a new generation of molecularly targeted anticancer drugs.

In this chapter we review the use of HTS for anticancer drug discovery and provide a general overview of an industrial screening operation. In particular, we focus on novel technologies and approaches that are helping to shrink screening timelines. Given that the bottlenecks in drug discovery have moved downstream of HTS in the screening pathway, we review new screening approaches that are being used to improve the drug-like quality of leads entering preclinical testing.

## ***1.2. Mechanism-Based Anticancer Drug Discovery***

Historically, drugs for the treatment of cancer were discovered through testing the cytotoxic effects of compounds on whole cells, organs, and even complete organisms. Subsequent work was required to elucidate the mechanism of action of the cytotoxic drugs. In contrast, modern anticancer drug discovery typically starts with the identification of a molecular target: either a protein or its RNA or DNA precursors. The strategic shift has been made possible by a dramatic improvement in our understanding of the molecular mechanisms underlying the pathogenesis of cancer.

The pharmaceutical industry's current strategy for controlling tumor growth selectively is to develop drugs that interfere with targets important in tumor angiogenesis, tumor invasion, tumor metastasis, cell cycle control, and apoptosis. Selection of individual targets for drug discovery is usually based on information obtainable from the academic literature or unpublished original research to which the pharmaceutical company has direct access. Once sequence information is known for a molecular target, bioinformatics can be employed to search for structurally related targets. Bioinformatics can uncover previously unknown proteins and thereby expand the number of potential targets that may require HTS for leads discovery.

The viability of a molecular target for drug discovery is based on a number of criteria that differ somewhat from one organization to another. In essence, for a target to be considered viable, some experimental validation is needed to show that interfering with its function will produce the desired effect *in vivo*. A number of strategies are generally employed to validate a target for drug discovery. The validation strategies include the use of gene knockouts, ribozymes, antisense technology, and RNA interference (RNAi). Gene knockouts in mice are particularly valuable since they will demonstrate the effect, in an *in vivo* setting, of abolishing the normal function of a gene product (1,2). However, gene knockouts also have several disadvantages for target validation; experimental timelines are long, costs are high, and the findings may relate more to developmental function than to adult function. As a consequence, the results of gene knockout experiments are sometimes difficult to interpret. Alternatively, antisense oligonucleotides or

ribozymes (RNA enzymes that cleave messenger RNA) can be used to study the effect of abrogating specific gene transcripts (3–5). RNAi is a new technology that uses gene silencing to manipulate gene expression in a number of cellular systems, including mammalian cells (6). RNAi can be employed in a high-throughput fashion to explore the function of a large number of putative molecular targets for anticancer drug discovery.

One caveat with the biological methods of validating a target, as described above, is that they do not always represent the effect that a small molecule has on a target. Moreover, it may not be worthwhile committing the time or resources into validating certain targets before proceeding to leads discovery. In some instances a pharmaceutical company may make the strategic decision to proceed with HTS against a nonvalidated target and to use the lead compounds themselves to demonstrate the effect of interfering with the target's function.

A research organization uses the approaches described above to create a list of potential targets for drug discovery. Further practical considerations are used to prioritize the targets for further consideration. The latter considerations include (1) the freedom to operate without infringing on intellectual property owned by another organization, (2) the feasibility of establishing a screening assay for discovering hit compounds and (3) the overall balance within the target portfolio of novelty (novel targets incur less competition from other pharmaceutical companies) versus validation (established pharmaceutical targets have undergone more experimental and clinical validation than novel targets and therefore present less risk of a negative outcome from drug discovery). An organization will often concentrate on certain classes of targets. For example, protein tyrosine kinases and G protein-coupled receptors (GPCRs) have received (and continue to receive) considerable attention as drug targets. Further efficiencies can be realized by employing the same assay technology for families of targets; this approach simplifies assay development and expedites HTS.

Table 1 outlines the cascade of screening steps that are taken in anticancer drug discovery for an enzyme target. The screening pathway begins with a high-throughput biochemical “primary” screen and the testing of a compound library of hundreds of thousands of compounds. The more advanced screening laboratories can easily achieve throughputs of 100,000 compounds or more per week. In general, it is usually possible to complete an HTS campaign, including the confirmation testing of hits, in less than 2 mo. Some simple measures are taken to remove nuisance or uninteresting compounds from the list of hits. A common first step is for an experienced chemist to review the structures and remove compounds that do not constitute good starting points for medicinal chemistry.

The compounds in a library are stored for HTS as solutions in dimethyl sulfoxide (DMSO); some compounds degrade during long-term storage. Purity testing is performed to address the possibility that a compound that was active in a screen was not the original structure. Liquid chromatography (LC) is employed, coupled with analysis by mass spectrometry (MS), to determine the structure or structures in the sample on the basis of compound mass. LC-MS can be performed in high throughput, which permits an entire set of a few hundred screening hits to be analyzed in less than a week.

Once the original hits have been selected, they are tested for potency in the primary high-throughput screen and ranked by their  $IC_{50}$  or  $EC_{50}$  values (concentrations that produce 50% inhibition or effective stimulation, respectively). The most potent com-

Table 1  
Anticancer Screening Pathway (Enzyme Target)

- 
1. In vitro screening
    - 1.1. HTS (inhibition of target in primary biochemical assay)
    - 1.2. Inhibition of target in cellular assay
    - 1.3. Inhibition of cell proliferation
    - 1.4. Selectivity assays
  2. In vitro ADME-Tox profiling
    - 2.1. Metabolic stability
    - 2.2. Cytochrome P450 inhibition
    - 2.3. Solubility
    - 2.4. Protein binding
    - 2.5. Cell permeability
  3. In vivo screening
    - 3.1. Rapid PK
    - 3.2. Full PK on selected compounds
    - 3.3. In vivo pharmacodynamic assay
    - 3.4. Tumor growth inhibition
- 

ABBREVIATIONS: ADME-Tox, absorption, distribution, metabolism, excretion, and toxicity; HTS, high-throughput; PK, pharmacokinetics.

pounds are assayed to determine whether they also inhibit the enzyme in a whole cell screen. At this juncture, the data are reviewed to decide whether there is a basis for allocating medicinal chemistry resources and continuing drug discovery.

If HTS has been successful in identifying potent hit compounds, a collaborative effort is initiated in which various chemical strategies are employed to synthesize compounds with improved inhibitory activity. Medicinal chemistry usually incorporates combinatorial approaches capable of generating large numbers of structurally related compounds; HTS is capable of delivering the throughput required for a timely analysis of the combinatorial libraries. The synthetic compounds are screened in the biochemical assay and the mechanistic cellular assay. Active compounds are also tested for their ability to inhibit proliferation of a tumor cell line in a functional screen. The selectivity of certain compounds for the target molecule may be explored using a panel of enzyme assays, which usually includes enzymes from the same family as the target molecule and may include unrelated enzymes.

Historically, the classical strategy taken for drug discovery was to focus initially on synthesizing compounds with maximal potency for the target molecule and to postpone testing of pharmacokinetic (PK) properties until efficacy testing in animal models was already under way. As a consequence, a high percentage of compounds with good potency for the target molecule turned out to show little efficacy in vivo because of poor PK properties, thus wasting many months or even years of research. It has also been argued that HTS strategies are biased toward the discovery of lipophilic compounds with inappropriate PK properties, especially when biochemical screening is employed (7). However, a paradigm shift toward optimizing PK properties has occurred in the last 5 yr. The modern approach is to eliminate compounds with poor PK properties early in the

drug discovery cycle and thereby improve the likelihood that lead compounds will be successful during *in vivo* testing. A panel of *in vitro* screens is used to profile the molecular properties of lead compounds and predict which compounds will have appropriate PK properties. The latter screens are usually run in parallel and include assays for metabolic stability, P450 inhibition, solubility, protein binding, and cell permeability. Computational approaches are also employed to predict which compounds possess properties in common with developmental drugs by calculating molecular properties such as molecular weight, numbers of hydrogen bond donors and acceptors, number of rotatable bonds, cLogP (calculated octanol-water partition coefficient; a predictor of lipophilicity), and polar surface area (PSA; a predictor of absorption) (7–9).

Compounds that demonstrate the most appropriate properties in the *in vitro* assays and computational models are advanced to the *in vivo* phase of testing. A favored approach is to test compounds initially in a high-throughput PK assay using a single dose in mice; only a small quantity of each compound is required. A caveat of the low-dose testing procedure is that it does not properly represent the PK that can be expected when the drug is given in higher doses or when it is formulated as a tablet. Higher throughputs can be achieved using cassette dosing techniques, in which each animal is dosed with several compounds or, preferably, pooling strategies in which plasma samples from several animals are combined before analysis (10). Only those compounds that achieve sufficiently high plasma concentrations and are cleared slowly will be advanced further down the discovery pathway. More extensive PK testing is carried out on compounds that achieve the selection criteria set for high-throughput PK testing. Two types of *in vivo* assay are commonly used for testing the anticancer activity of lead compounds. Pharmacodynamic assays are used to measure the efficacy of a compound in inhibiting the target enzyme in tumors, whereas a tumor growth inhibition (TGI) assay measures the efficacy of a compound in inhibiting tumor growth. Compounds that show excellent efficacy in preventing disease progression in the animal model will be considered for nomination as clinical candidates.

### ***1.3. Leads Discovery***

HTS is best considered as one of several approaches that can be employed to find lead compounds for drug discovery. One alternative approach to HTS is structure-based drug design, which employs computer modeling to design compounds that bind to regions of the target molecule known to be important for its normal function (11). A three-dimensional structure of the target molecule is a prerequisite for structure-based design. The molecular coordinates of the target structure can be obtained either by X-ray crystallography or by homology modeling. In the latter approach, the primary sequence of a novel protein is compared with the sequence of a protein whose structure is already known using software that is able to predict the novel structure. A caveat of homology modeling, however, is that it fails to consider the flexibility of protein conformations; therefore, the homology models may not always be accurate.

A second alternative approach to HTS is to initiate a drug discovery project based on a compound with published activity against the target. If freedom to operate on the published structure is restricted by intellectual property, a pharmaceutical company will sometimes adopt medicinal chemistry approaches to discover similar compounds that have activity against the target, yet are not covered by any patent.

In practice, most organizations use a flexible research strategy for discovering lead compounds. Whenever feasible, HTS is supplemented with structure-based drug design and/or medicinal chemistry approaches based on published structures to increase the likelihood of finding successful lead compounds.

An emerging innovation for leads discovery is to make strategic use of advances in high-performance computing platforms. *Virtual screening* is used to expand the screening of structures beyond those compounds existing in an organization's physical inventory (11–13). A virtual library is created containing compounds in vendors' compound collections, compounds in the screening library, and even compounds created computationally that are not available from any known source. By expanding structure-based drug design to virtual libraries of compounds held in huge databases, millions of virtual compounds can be “docked” computationally against the target molecule in just a few days. Computational scoring methods are used to identify those compounds that bind tightly to the target. The virtual hits are subsequently purchased from vendors or synthesized in-house if no commercial source can be found. Virtual screening is also used to identify those compounds in the screening library that are most likely to be hits. When used tactically in this way, virtual screening can accelerate lead identification and reduce costs. As a consequence, some companies have installed industrial-scale “cherry-picking” systems that allow focused collections of a few thousand compounds, identified as potential hits by virtual screening, to be selected quickly from the library and compiled into plates for subsequent in vitro screening.

## 2. HIGH-THROUGHPUT ASSAY METHODOLOGIES

Knowledge of the molecular target and expertise in assay design are important prerequisites for developing a high-throughput screen. HTS assays can be run using either cells or purified molecular targets. Cell types can be mammalian, yeast, or bacterial. Cells are engineered to overexpress a target protein or are transfected with a reporter gene. The criteria used in deciding which assay technology to use for HTS include sensitivity, speed, ease of automation, reliability, safety, and cost.

Early high-throughput screens relied on measurements of absorbance and radioisotopes. Luminescent assay techniques were subsequently exploited, most notably for reporter gene assays (14). More recently, fluorescence detection has gained prominence for HTS because of its high sensitivity, versatility, and compatibility with assay miniaturization (15). Fluorescence-based screening techniques include prompt fluorescence intensity, time-resolved fluorescence (TRF), fluorescence resonance energy transfer (FRET), and fluorescence polarization (FP).

### 2.1. Homogeneous and Heterogeneous Assay Technologies

The assay design will impact both the timeline required to develop a screen and the screening throughput. A simple screen with few operational steps can be optimized quickly and is very amenable to automation. Homogeneous assays (e.g., FRET and FP) have become increasingly popular for HTS: they use “mix-and-measure” methods that avoid separation steps and provide excellent precision. Heterogeneous assays (e.g., enzyme-linked immunosorbent assays [ELISAs]) require separation steps that go beyond simple fluid additions, incubations, and readings to include manipulations such as washing steps, filtration steps, or plate-to-plate transfers. Despite the operational limitations

**Table 2**  
**Protein Kinase Assay Methodologies Commonly Used for HTS**

<i>Assay Methodology</i>	<i>Assay type</i>	<i>Assay readout</i>	<i>Source</i>
DELFI <sup>®</sup>	Heterogeneous	Time-resolved fluorescence	Perkin Elmer Life Sciences (Boston, MA)
ELISA	Heterogeneous	Absorbance	Multiple
ALPHAScreen <sup>™</sup>	Homogeneous	Fluorescence	Perkin Elmer Life Sciences
FlashPlate <sup>®</sup>	Homogeneous	Scintillation (radiometric)	Perkin Elmer Life Sciences
FP	Homogeneous	Prompt fluorescence	Multiple
FRET	Homogeneous	Prompt or time-resolved fluorescence	Multiple
SPA	Homogeneous	Scintillation (radiometric)	Amersham Biosciences (Piscataway, NJ)

ABBREVIATIONS: DELFIA<sup>®</sup>, dissociation-enhanced lanthanide fluoroimmunoassay; ELISA, enzyme-linked immunosorbent assay; ALPHAScreen<sup>™</sup>, amplified luminescent proximity homogeneous assay; FP, fluorescence polarization; FRET, fluorescence resonance energy transfer; SPA, scintillation proximity assay.

of heterogeneous assays for HTS, they do offer some advantages. One advantage of heterogeneous assays is that compounds are not present when the signal is measured, which obviates the possibility that compounds will interfere with the assay readout. A second advantage of heterogeneous assays is that they typically generate a higher signal-to-background ratio.

HTS methodologies have evolved enormously over the past decade. For example, when HTS was in its infancy, tyrosine kinases were commonly screened by measuring the incorporation of <sup>32</sup>P into substrate from radiolabeled ATP or by using ELISAs employing anti-phosphotyrosine antibodies. An adaptation of ELISA methodology—DELFI<sup>®</sup> (dissociation-enhanced lanthanide fluoroimmunoassay)—which employs a TRF readout, has also been used (16). The above screening methods require a separation step before measuring the assay signal. More recently, homogeneous assay technologies such as SPA (scintillation proximity assay), FP, FRET, ALPHAScreen<sup>™</sup> (amplified luminescent proximity homogeneous assay), and scintillating microplates (FlashPlate<sup>®</sup>) have all become available for HTS (15–20). Table 2 lists the assay methods that are commonly used to screen for inhibitors of protein kinases. Many of the more novel assay technologies have been commercialized for HTS and are easy to use; in some cases the vendors offer kits for HTS. Consequently, assay development for kinases and certain other target classes can be reduced down to a simple set of guidelines, which enables new screens to be developed and transferred to HTS in a rapid and streamlined process. Stocks of generic screening reagents can sometimes be used for multiple screens, which can further accelerate assay development and screening.

## **2.2. Cell-Based and Biochemical Screens**

Screening for inhibitors of novel biological targets is often performed using a well-characterized biochemical assay established with purified components. The biochemical

assays typically examine a single biochemical event such as an enzymatic reaction or a binding interaction. With enzyme targets, most of which are intracellular, a biochemical assay is usually preferred for HTS. Cell-based HTS is sometimes preferred for targets expressed within the cell membrane and for screening against several targets in a pathway simultaneously. The complexity of cell-based screens, compared with most biochemical screens, usually results in less reproducible screening data. For a biochemical screen, large stocks of reagents can be prepared and frozen down for the entire screening campaign, which simplifies the screening operation. However, for some targets, it may be impractical to produce and isolate large quantities of specialized reagents (such as a membrane-bound receptor), and a cell-based screen becomes more attractive for HTS than a biochemical assay. For example, yeast-based reporter gene assays offer a cheap and straightforward alternative to biochemical methods for screening against GPCRs (21). Since cell-based and biochemical assays each offer advantages for certain targets, it is usually advisable for HTS laboratories to use both approaches for leads discovery.

### ***2.3. Advanced Fluorescence Assay Technologies***

Advances in imaging and assay technologies have led to the emergence of high-content screening (HCS), which yields far more information on cells than single-measurement screens (22). Through HCS, changes in cell shape and size, changes in the distribution of intracellular proteins, and multiple cellular targets can be assayed simultaneously through judicious selection of fluorescent probes (23). An example of the use of HCS is for measuring apoptosis, in which changes in nuclear morphology, cytoskeleton organization, and mitochondrial physiology are measured in a multiparameter fashion (24). A caveat with HCS is that it can generate huge quantities of data, presenting a data analysis challenge that has tended to limit its application to screens downstream of HTS in the drug discovery pathway.

Another advanced fluorescence assay technology involves the use of confocal laser techniques to measure the fluorescence properties of single molecules as they move through tiny volumes as small as 1 fl (25). The best known example of a single-molecule detection (SMD) assay is fluorescence correlation spectroscopy (FCS). Because the detection volume for SMD assays is so small, they are ideally suited to miniaturization and have found application in the densest plate formats, including 1536-well plates.

## **3. AUTOMATION FOR HIGH-THROUGHPUT SCREENING**

There has been tremendous growth in the number and variety of robotic instruments specifically designed for automated drug discovery. A research organization now has a variety of options for equipping the HTS laboratory according to the prevailing types of assays, the required throughput, and the available budget (Table 3).

The early history of HTS was characterized by the use of fully integrated robotic screening systems that incorporated a method of moving assay plates from station to station, generally a robotic arm located centrally or on a linear rail. A drawback of integrated robotic systems is that many months are required to design, build, and test the systems. Deployment of integrated robotic systems is quite slow compared with workstations, which can be installed quickly in the laboratory and which require less testing before becoming operational.



**Table 3**  
**Equipment Platforms for HTS**

<i>Parameter</i>	<i>Manual devices</i>	<i>Workstations</i>	<i>Laboratory robotics</i>	<i>Industrial robotics</i>
<i>Cost</i>	Low	Medium	High	Very high
<i>Throughput capability (tests/d)</i>	5000	50,000	75,000	250,000
<i>Advantages</i>	Cheap	High throughput, flexible, quick to deploy	Suited to heterogeneous assays, unattended operation	Capable of uHTS, component of drug discovery factory
<i>Disadvantages</i>	Low throughput, subject to human error	Requires human intervention, low throughput for heterogeneous assays	Expensive, slow to deploy, difficult error recovery	Very expensive and complex, dedicated facility

HTS, high-throughput; uHTS, ultra-high-throughput.

Two of the most important recent advances for HTS have been the development of homogeneous assay formats and an increase in the use of workstations for screening. The use of homogeneous fluorescence assay technologies has resulted in assays that require fewer steps and thus less plate manipulation. Liquid-handling workstations, with the capability of moving large numbers of plates on tracks, can be very productive when applied to homogeneous assays (an example is the MiniTrak™ from Perkin Elmer Life Sciences, Boston, MA). Plates can be transferred manually from the liquid-handling workstation to an incubator or reader by using removable plate stackers.

Integrated robotic systems still have a role to play in screening, especially when complex assays involving multiple steps are involved (e.g., ELISAs or cell-based assays). Moreover, robotic systems can run for long periods unattended and are capable of achieving high throughputs by functioning during periods when laboratory personnel are not normally in attendance. It is therefore easy to understand why traditional robotic systems are still commonplace in most HTS facilities.

A more recent trend, which has found most resonance with the largest pharmaceutical companies, is toward industrial-scale automation that is compatible with the vision of a drug discovery factory (26). When applied to HTS, the approach is to build very large robotic systems capable of achieving throughputs well in excess of 100,000 tests per day; this is the realm of ultra high-throughput screening (uHTS).

In the case of the traditional robotic systems, the robotic arm tends to be in constant demand while many of the individual equipment modules stand idle, awaiting delivery of plates. In short, the robotic arm can become a bottleneck in the system, preventing higher levels of productivity from being attained. Industrial-scale robotic systems (such

Table 4  
Impact of Assay Miniaturization on Reagent Costs and Screening Throughput

<i>Plate density (wells/plate)</i>	<i>Assay volume (<math>\mu</math>L)</i>	<i>Throughput (tests/d)</i>	<i>Reagent costs/well (\$)</i>
96	50–200	10,000	0.50
384	20–50	40,000	0.20
1536	2.5–10	60,000	0.05

as those supplied by The Automation Partnership [Royston, UK] and RTS Thurnall [Manchester, UK]) have solved the productivity problem by managing to keep all components of the robotic system working continuously. Plates progress along tracks at a constant pace, instead of being moved individually by robotic arms. When there is potential for one step of an assay to hold up the progress of plates along the system (e.g., when a plate needs to be subjected to three cycles of washing) an engineering solution is found to minimize the impact on overall productivity (e.g., using three washers, one for each washing cycle). The factory-style approach to HTS is enabling large pharmaceutical companies to keep pace with the numbers of targets that have been unleashed by functional genomics.

#### 4. SCREEN MINIATURIZATION

There has been a trend in the industry over the last 5 yr toward high-density plate formats and low-volume assays. Miniaturization has been driven by a desire to reduce reagent costs, to conserve compound libraries, and to allow for uHTS by enhancing throughput (27). The benefits of screen miniaturization are illustrated in Table 4. The greatest increase in throughput comes when switching from 96-well to 384-well plates. Screens that would take weeks or months to complete in 96-well plates can be carried out in weeks or days in 384-well plates.

The equipment required to perform 384-well HTS is well proven and is available at reasonable cost from many different manufacturers. In fact, the introduction of the 384-well format has been so successful that more than half of all HTS was performed in 384-well plates in 2001 (28). Specialized equipment is required to perform HTS in volumes below 5  $\mu$ L. Miniaturizing a screen to run in a 1536-well plate can greatly reduce costs, but custom-designed liquid handling becomes a necessity. The technological challenges posed by 1536-well HTS probably explain why less than 4% of all HTS was performed in 1536-well plates in 2001 (28).

Low-volume dispensing technologies can be divided into two classes: noncontact dispensing and contact dispensing. Cartesian Technologies (Irvine, CA) has long been a leader in noncontact dispensing; its SynQuad™ technology can accurately dispense volumes as low as 50 nL very rapidly. Equipment introduced more recently (e.g., the Spot-On™ technology from Allegro Technologies, Dublin, Ireland) promises improved accuracy at even lower volumes. Contact dispensing involves the use of pin tools that were originally developed for generating DNA microarrays; volumes as low as 5 nL can be handled in this way. Contact dispensing has proved to be very useful for transferring compound libraries to high-density plates.

## 5. COMPOUND LIBRARY MANAGEMENT

It is axiomatic that the hits generated from an HTS campaign will only be as good as the compound library that was screened. Over the past few years a number of changes have taken place to enhance the quality of screening collections. Many discovery organizations have retreated from screening natural products because of the costs and timelines involved in isolating and identifying active compounds from natural mixtures.

When it comes to synthesized compound libraries, the emphasis is now more on quality than quantity. Companies are increasingly using computational methods to select compounds for their collections, either to increase diversity or to generate sublibraries for specific target classes. The increasing reliance on more focused compound collections has obviated the need to screen huge compound libraries against every target.

Compound handling has also benefited from innovations in robotics. Companies such as RTS Thurnall, The Automation Partnership, and REM (Oberdiessbach, Switzerland) can supply fully automated compound stores. The stores can hold millions of compounds in a variety of formats including standard vials, plates, and minitubes. The storage units are incorporated into environmental enclosures with temperature and humidity control. Integrated liquid-handling stations generate compound plates preformatted for HTS. An innovation that has greatly aided focused screening efforts is the development of 96-well minitubes that can be tracked individually. Compounds in minitubes are managed robotically within automated stores; thousands of compounds can be cherry-picked and transferred into screening plates within a 24-h period.

## 6. HIGH-THROUGHPUT ADME-TOX ASSAYS

Discovery teams are now tasked with generating information on the ADME-Tox properties of promising compounds. This has led to a requirement for higher throughput ADME-Tox assays that can be automated for testing compounds early in the discovery pathway (29).

### *6.1. High-Throughput ADME Screens*

The solubility of a compound is critical to its bioavailability. A number of automated assays have been developed to measure compound solubility, but the method most amenable to high-throughput applications is probably the laser nephelometry method that can be performed in 96-well plates (30). Laser nephelometry involves passing a polarized laser beam through a compound solution to measure light scattering caused by precipitated compound.

Orally available drugs have to be able to get into the bloodstream through the gut epithelium. Small rodent animal models can be used for testing compound absorption, but are not suitable for high-throughput applications. In vitro methods have been developed that utilize both cultured cell monolayers and artificial membranes. The cell-based absorption assays most commonly use Caco-2 cells (28), but Madin-Darby canine kidney (MDCK) cells are also used. The cells are grown in a monolayer on a membrane built into an insert in a 24-well plate. The cells form tight junctions; compound added to the apical compartment can only get to the basolateral compartment through the cells. The amount of compound in the basolateral medium, as measured by LC-MS, can be used to calculate an apparent permeability coefficient ( $P_{app}$ ) using Artursson's equation (31). Artificial

membrane assays are run in a similar manner to the cell-based absorption assays but use an immobilized artificial membrane in place of the cell layer. One example is the parallel artificial membrane permeability assay (PAMPA) that can be carried out in the 96-well format (32).

Drug stability can be assayed using cultured human hepatocytes, human microsomes, or animal hepatocytes (29). Tissue from human sources is expensive and can create safety concerns, but the use of animal hepatocytes may not provide a good reflection of the rate of drug metabolism in humans. Drug metabolism in metabolic stability assays is generally monitored by LC-MS (10).

Screens for cytochrome P450 (CYP) inhibition are widely used in drug discovery to eliminate compounds that might cause drug-drug interactions. Commercially available assays for CYP inhibition exist that are easily automated (33).

### 6.2. High-Throughput Toxicity Screens

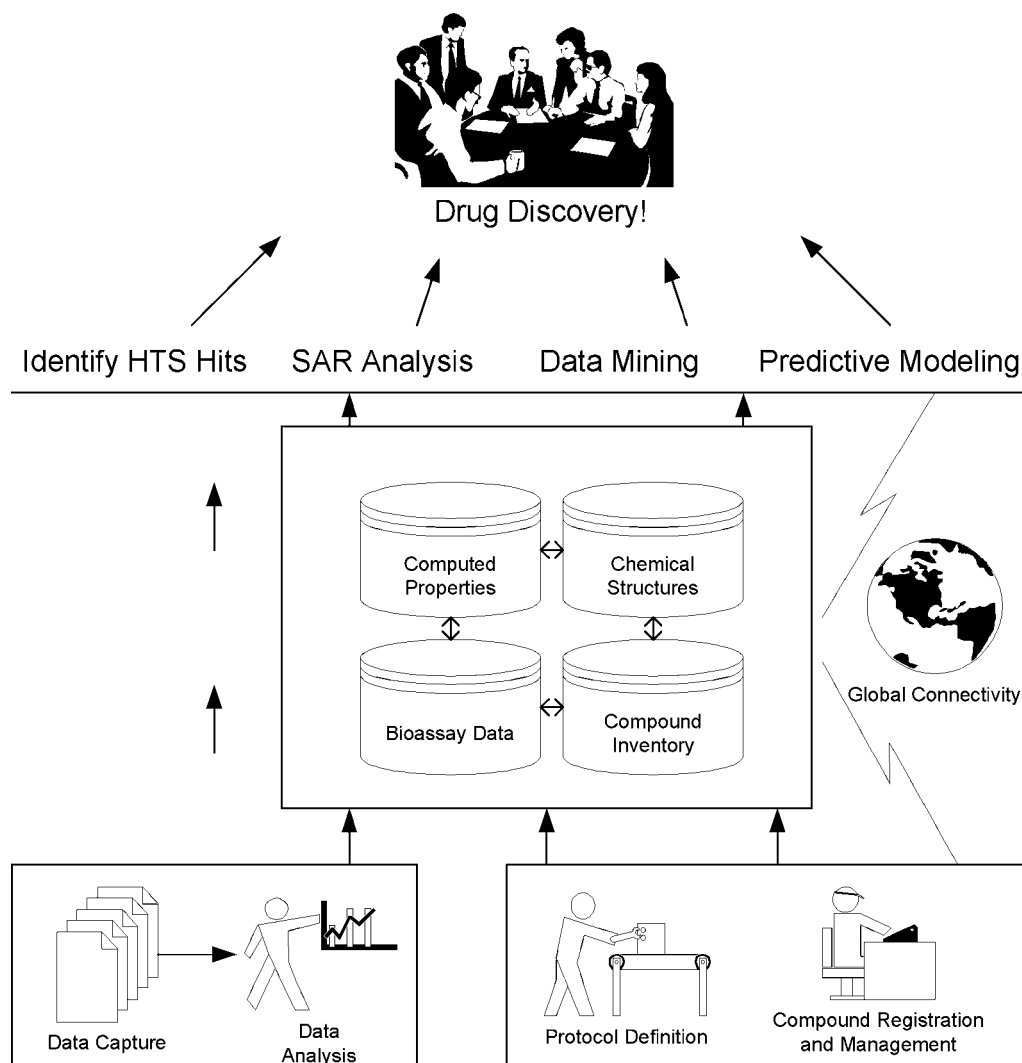
The simplest measure of a compound's toxicity is to test its effect on the viability of cultured cells. Since most *in vivo* toxicity is a reflection of hepatotoxicity, human hepatocyte cell lines are frequently used. Cell viability can be measured using an assay that measures the color change when 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) is metabolized by the mitochondria of living cells. An alternative cell viability assay measures cellular ATP content. Both the MTT assay and the ATP assay are easily automated (34).

Genotoxicity tests use specifically engineered strains of *Salmonella typhimurium* that can be used to screen for mutagenic compounds (35). The assay detects a change in pH; a spectrophotometer measures the color change indicated by a pH-sensitive dye in the culture medium. The advent of DNA microarrays and other genomics technologies has allowed the simultaneous monitoring of transcripts from thousands of genes known to be involved in responses to toxic insult (36). The latter genes include heat shock genes, cytochrome P450s, and glutathione-S-transferase (GST). Transcription profiling enables discovery groups to build a detailed genotoxic profile on compounds of interest.

Mutations in the hERG gene, which encodes a cardiac potassium channel, can cause long Q-T syndrome (LQTS), which can lead to sudden death induced by cardiac arrhythmia (37). Pharmacological blockade of the hERG channel can result in a similar cardiac effect; consequently there is much interest in screening for hERG channel effects. The traditional electrophysiological hERG channel assays such as patch clamping do not have sufficient throughput, so a number of higher throughput assays have been developed for drug discovery (38). Two assays use fluorescent voltage-sensitive dyes; they measure a change in membrane potential when cells transfected with the hERG channel are treated with hERG channel blockers. A third assay uses atomic absorption spectrometry to measure the efflux of rubidium ions from hERG-transfected cells.

## 7. DATA MANAGEMENT FOR HIGH-THROUGHPUT SCREENING

Since so much information is generated during a drug discovery program, a good data management system is indispensable to the pharmaceutical organization. The data management system is used for capturing and storing data that are generated at different stages of the discovery pathway and for making the data available to the multidisciplinary discovery teams. Research groups use the data management system to make informed



**Fig. 1.** A data management system for drug discovery. The discovery data are held in a series of interfaced Oracle<sup>®</sup> databases. Mechanisms exist to streamline the entry of data into the databases and to control data quality. A variety of software tools are used to derive knowledge from the data for drug discovery. HTS, high-throughput screening; SAR, structure-activity relationship.

decisions about which series of compounds to synthesize and which compounds to advance down the discovery pathway. It is quite common for some research organizations to be distributed across different countries and different time zones. A company's data management system can therefore play a vital role in coordinating the activities of geographically distributed groups. The data management system acts as a central resource that collects and collates data in real time and organizes the information for dissemination to researchers across the organization.

A typical data management system for drug discovery is illustrated in Fig. 1. A database or series of interconnected databases lies at the heart of the system. Most systems

utilize the Oracle® database (Redwood Shores, CA), which facilitates system integration and data exchange between different products and employs technologies that enable deployment through client-server and web-based technologies. The compound inventory system records the corporate identifier, location, and other critical information (e.g., batch details) about each compound. Structural information is usually maintained in a commercial chemical database (e.g., MDL® ISIS/Host from MDL®, San Leandro, CA) that permits compound searches based on chemical substructures. Many companies have built their own databases to store bioassay and compound inventory information, but commercial options also exist. The modern data management system includes a large database for storing multiple physiochemical properties that are computed for each compound held in the inventory.

The data management system includes mechanisms to assist the flow of data into the databases, to regulate quality, and to enforce the business rules of the organization. Database protocols (metadata) describe the assays and are established for each screen. A compound registration system is used to enter structural and other critical information about each compound that is received into the inventory. Researchers submit compound requests using an application that accesses the compound inventory database to report the availability of individual compounds and to control access to restricted samples (not shown in Fig. 1). A data capture application is necessary to process large volumes of data rapidly and to streamline their entry into the database. Software applications are used to analyze the assay data and to detect aberrant results as part of standard quality control (QC) procedures.

Data stored in the database do not directly translate into knowledge that can be used by the drug discovery teams. A variety of software applications are used to query the database and to report results that are important to the decision-making process for drug discovery. In an environment of rapid change, expansion, and merger, it is considered best practice to build data management systems that facilitate multisite access and good performance at remote locations. A combination of client-server and web-based technologies for querying the database will generally achieve the correct balance between application functionality and ease of deployment.

The archived data form the basis for future reporting by scientists throughout the company. Data may also be collected and reorganized into a data warehouse to improve query and reporting efficiency (not shown in Fig. 1). An obvious example of a database query is one used to identify hits from HTS. Further down the discovery cascade, structure-activity relationships (SAR) are built by querying the database. SAR analyses combine biological and chemical information and are used to identify the structural features of compounds that are important for biological activity against the target molecule. SAR analyses are also used to determine the chemical features that produce undesirable pharmaceutical effects. Data mining is a strategy for analyzing large quantities of data held in databases to reveal trends and relationships that would otherwise remain hidden. Data mining software is frequently employed to look for trends in HTS data: to identify hits that would be overlooked by conventional data analysis and to perform sophisticated QC analyses. Modeling techniques, using data acquired from a limited screening “training set,” can be used to predict other compounds in the screening library that are likely to be hits. Predictive modeling, when combined with industrial cherry-picking capabilities and a sequential approach to screening, can be used to identify useful hits without needing to screen the entire compound library (39).

## 8. CONCLUSIONS

The alternative approaches to drug discovery notwithstanding, most biopharmaceutical companies have adopted HTS as a cornerstone of their leads discovery operation. Indeed, it is fair to say that HTS has established itself over recent years as an essential component of drug discovery. However, the role of HTS laboratories is changing. At the start of the HTS revolution, screening against a drug target to find hits required many months or even years of concentrated effort. As the field has progressed, advances in assay methodologies, miniaturization, high-density plate formats, and screening automation have resulted in a dramatic reduction in the cycle time for HTS, which is now completed in a matter of a few weeks. Consequently, in most pharmaceutical companies, the primary screen is no longer seen as the bottleneck in drug discovery.

At the current time, business imperatives in the pharmaceutical industry are driving program managers to shorten research timelines and discover increasing numbers of clinical candidates. Research efforts are focusing on how to identify high-quality lead compounds that are less likely to fail in preclinical testing and HTS laboratories are ideally placed to assist in this regard. Much of the infrastructure that has been established for running a primary screen at high throughput (notably laboratory automation, assay methodologies, and data management systems) is being applied to downstream needs in drug discovery. Many HTS departments are shouldering the responsibility for running preliminary ADME-Tox screens, and the trend toward automating assays that have traditionally been run in low throughput is likely to continue. Some companies are even taking the automation of drug discovery to the extreme, with the establishment of factory-like facilities.

Many companies cannot afford to screen their entire compound library against all the targets proposed for HTS. It is a measure of how far screening laboratories have progressed that logistical concerns no longer dominate decisions over how many primary targets to investigate by HTS. Miniaturization has reduced the cost of individual screening campaigns, but the flood of new targets produced by genomics approaches (for which HTS is often the only viable option for leads discovery) has driven running costs even higher. It is partly as a consequence of the financial considerations that, in the future, we are likely to witness a symbiotic relationship forming between HTS (in vitro screening) and virtual (*in silico*) screening. HTS and virtual screening are complementary approaches for leads discovery: they can be employed independently of each other or in combination, both methodologies play the “numbers game,” and both ultimately require large sets of compounds to be screened in vitro. In the anticancer field, HTS (both of random libraries and focused compound collections) will continue to play a pivotal role in mechanistic discovery as high-throughput approaches are increasingly applied to accelerate the identification of new clinical drug candidates.

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