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REVIEW: HISTORICAL PERSPECTIVES IN PHARMACOLOGY

Origin and evolution of high throughput screening

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This article reviews the origin and evolution of high throughput screening (HTS) through the experience of an individual pharmaceutical company, revealing some of the mysteries of the early stages of drug discovery to the wider pharmacology audience. HTS in this company (Pfizer, Groton, USA) had its origin in natural products screening in 1986, by substituting fermentation broths with dimethyl sulphoxide solutions of synthetic compounds, using 96-well plates and reduced assay volumes of 50-100μl. A nominal 30mM source compound concentration provided high μM assay concentrations. Starting at 800 compounds each week, the process reached a steady state of 7200 compounds per week by 1989. Screening in the Applied Biotechnology and Screening Group was centralized with screens operating in lock-step to maximize efficiency. Initial screens were full files run in triplicate. Autoradiography and image analysis were introduced for ^{125}I receptor ligand screens. Reverse transcriptase (RT) coupled with quantitative PCR and multiplexing addressed several targets in a single assay. By 1992 HTS produced 'hits' as starting matter for approximately 40% of the Discovery portfolio. In 1995, the HTS methodology was expanded to include ADMET targets. ADME targets required each compound to be physically detected leading to the development of automated high throughput LC-MS. In 1996, 90 compounds/week were screened in microsomal, protein binding and serum stability assays. Subsequently, the mutagenic Ames assay was adapted to a 96-well plate liquid assay and novel algorithms permitted automated image analysis of the micronucleus assay. By 1999 ADME HTS was fully integrated into the discovery cycle.
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Abbreviations: ADMET, adsorption, distribution, excretion and toxicology; cAMP, cyclic adenosine monophosphate; CETP, cholesteryl ester transfer protein; DMF, dimethylformamide; DMSO, dimethyl sulphoxide; HTS, high throughput screening; LDL-R, low-density lipoprotein receptor; MTT, (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; MTTP, microsomal triglyceride transfer protein; PDE IV, phosphodiesterase IV; RT, reverse transcriptase; SAR, structure–activity relationships; TPX, 4-methylpentene-1copolymer

Introduction

The wide acceptance and universal application of high throughput screening (HTS) in the Pharmaceutical and Biotechnology industry, along with recent expansion into the academic realm belies its modest initial debut. HTS provides a practical method to investigate large numbers of synthetic compounds in miniaturized *in vitro* assays to identify those capable of modulating the biological target of interest. Before its inception traditional biochemical and pharmacological drug discovery methods required 1 ml reactions in individual test tubes and weighed dry compounds that were dissolved in various vehicles to provide true solutions. This approach limited assay capacity for a laboratory to 20–50 compounds per week. Given these constraints a 'Prototype File' of approximately 3000 selected compounds representing structural diversity could be screened for new target modulators in 1–2 years. The advent of R DNA with access to new therapeutic targets quickly revealed the inadequacy of such limited screening to identify structural prototype hits capable of modulating these new targets. This drug discovery singularity set the stage for HTS as a practical method to screen several hundred thousand compounds against new targets rapidly and cost effectively. The state of HTS in the early years, together with questions, options and strategies is broadly captured in an interview by Sills (1998). At this stage HTS costs were determined to be acceptable within each company and no comparative data were available until several years later. The perceived sensitivity of this information precluded direct publication and all comparisons were by external surveys (Fox *et al.*, 2001) or consultants reviews (Beggs, 2000).

This account is a personal perspective of the origin of the HTS concept at Pfizer Global Research and Development: its early evolution covering the period 1984 through 1995,

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followed by its conceptual application to drug metabolism and toxicity screening from 1995 through 2000. This is presented in three distinct phases: (a) development of the concept and philosophical choices with regard to implementation, (b) practical implementation and technology development and finally, (c) the logical expansion to include related disciplines in drug development. It is primarily a story of ideas, people and decisions based on scientific principles as it was for many years considered a competitive advantage precluding any publication of results other than internal proposals and white papers. We are indebted to many colleagues who contributed suggestions and ideas to enhance the initial concept thereby rendering it a practical process and they are recognized in the acknowledgments. The form of this narrative will be a philosophical perspective intertwined with the early development of the technology and the infrastructure necessary to convert concept to reality. As this is not a formal review of HTS it intentionally omits many more recent key developments in automation, liquid handling, informatics and novel assay formats. These omissions are competently reviewed by experts in each area in several excellent books that appeared subsequent to the 1995 ending of this account for HTS. The first by Devlin (1997) followed by Seethala and Fernandes (2001), Janzen (2002) and Minor (2006) provide thorough and extensive coverage of many aspects of HTS within drug discovery.

Genesis of the HTS concept and philosophical choices at Pfizer

The project that initiated this adventure was an attempt in the Molecular Genetics Group to exploit R DNA to create novel antibiotics or improve antibiotic production. Success in cloning and constructing appropriate Streptomycete libraries highlighted the real challenge: how to identify antibiotic production in large libraries of 10–100 000 clones that moreover required screening under a variety of fermentation conditions? Pfizer's Natural Products Screening Group located in Nagoya, Japan, was the logical choice for this task as an extension of their current discovery role. Actinomycetes, primarily recovered from soil samples, were cultured under a variety of conditions and the fermentation broths assayed for biological activity against a panel of microorganisms. This was a manual process that used 10 ml fermentation tubes and had a maximum capacity of approximately 200 new soil samples per week, with two isolation conditions and two fermentation broths for each yielding a total of 800 fermentation broths each week for screening. Filter paper disks were used to convey fermentation broths to bacterial lawns to assess antibiotic production by way of zones of inhibition. This process was clearly inadequate to support efficient screening of the large R DNA libraries while maintaining routine discovery support.

Screening capacity needed to increase at least 10-fold and become more facile. Our familiarity with 96-well microtitre plates offered a potential solution. The ability to process 96 fermentations in parallel had several immediate advantages: (a) a fixed format with a single lid providing access to 96 samples simultaneously, (b) compatibility with 8 or 12 channel pipettes and (c) reduced incubation space increasing capacity. A series of experiments using known antibiotic-producing actinomycetes proved the feasibility of this format but, although successful, the 100 μl volumes were insufficient to support all discovery screening programs. We proposed automating natural product screening using a custom 24-well plate format. This fixed format fermentation plate permitted the entire process to be automated using robotics. A series of systems were developed for dispensing fermentation media into plates, inoculating them with actinomycetes, aspirating and dispensing 25 μl drops of the fermentation broths on to the surface of agar plates seeded with bacterial indicators. Together, these systems dramatically increased fermentation capacity. Delivery of samples as drops directly on to seeded bacterial lawns was a major difference from the established methods using filter discs or cutting wells in the agar. However, this simple change increased speed, decreased labour and facilitated automation of the process.

A key scientific development during this time was the discovery of the structural and functional homology of human and yeast *ras* oncogenes (Powers *et al.*, 1984; Kataoka *et al.*, 1985). A collaboration with Michael Wigler at Cold Spring Harbor Laboratories was established to assess oncogenes as targets in preference to general cytotoxic agents. This was an ideal test case for the automated natural products system as it used a differential panel of yeast strains with specific genes and deletions to distinguish between general cytotoxic agents, cyclic-amp inhibitors and *h-ras* normal and mutant specific inhibitors as simple zones of inhibition.

We believed that the key to successful screening was finding the balance between number and diversity of samples screened with respect to both organisms and fermentation conditions. Therefore the system was designed to process and assay 10 000 fermentation broths per week. The automation project started in 1984 and was completely implemented in 1990 in Nagoya, Japan.

Natural products screening, having multiple fermentation conditions, is implicitly high throughput. A key attribute is that the substances of interest are available as solutions, that is, fermentation broths ready for assay. Although it was a simple conceptual step to replace the fermentation broths with chemical compounds, the challenge was to provide these synthetic compounds as solutions ready for assaying. The number and diversity balance principle was fulfilled by using the file of synthetic compounds generated over decades of synthesis and its attendant divergent structure–activity relationships (SAR), as chemical space was explored in the pursuit of potency and selectivity for diverse targets. Furthermore, we were confident that we had the ability to screen 10 000 samples per week as that was the design parameter for the automated screening system at Nagoya. This was the origin of HTS in Pfizer in 1986 as a powerful, rational yet empirical approach to identify molecules that modulated targets of therapeutic interest. Nonetheless, with its bold claim to be able to identify prototype lead molecules by increasing screening capacity by 50 to 100-fold while simultaneously decreasing staffing by at least 50%, this divergence from current screening practices was met with a healthy and robust scepticism. However, scientific

scepticism was placed in perspective when individual scientists acknowledged a belief that most assays except their own would be compatible with HTS. Table 1 contrasts the processes and lists the changes that were introduced.

A conceptual advantage of screening synthetic compounds was that the primary HTS activity could be correlated with compound structure thereby deriving some structural trends as a surrogate for traditional SAR. Another conceptual strength of HTS was that as more assays were executed the 'hits' could be compared and some indication of selectivity would become apparent to guide selection of hits to follow-up.

In contrast to the simplicity of the concept, the practical implementation exposed many philosophical alternatives that have been debated to this day with distinct variations being practiced. We will describe some of the paths that were explored and implemented during the early years of HTS and point out areas where the same dilemmas still exist. (Figure 1).

Compound access

All companies have collections of compounds synthesized over decades as SAR diverged for active substances. These compounds were generally stored as solids in a company archive. Traditional pharmacology used 5–10 mg of compound and required considerable time to coax this into solution using a variety of interesting cocktails. Weighing the compounds became the first rate-limiting step to accessing them for HTS.

Our experience with fermentation broths led to the observation that active metabolites were detectable despite the fact that they were often not the major component of a fermentation broth and their concentration was variable. Successful assays were dependent on attaining a minimal threshold of activity. This notion meant that assay success could be enhanced by increasing compound concentration above the minimal definition of an acceptable hit (while debated vigorously it was usually defined as low μM). The dynamic range of the assay concentration also allowed us to be less rigorous in the 'weighing' of compounds. For practical purposes we initially relied on a visually estimated (flicked) dispensing of $\sim$1 mg of compound. Consider the case that a 'flicked' sample varied by a maximum of 5-fold and the chosen screening concentration was 30 μM, while 'hits' were defined as active at 1 μM. The actual screening concentration could therefore vary from 6 to 150 μM always exceeding the target concentration of low μM. Initially, compounds were provided at a rate of 200/week, increasing to 800/week and then 1440/week in 1987 as HTS became accepted and utilized. Since compounds were supplied by both Groton (USA) and Sandwich (UK) sites, this amounted to a screening capacity of 2880 newly 'flicked' samples per week. New screens could access both dimethyl sulphoxide (DMSO) stocks as well as new compounds thereby increasing the attainable weekly screening rate. Once the system was established and successful, it became clear that recording the weight of 'flicked' samples on a balance added only $\sim$10% time overhead in compound dispensing. This resource increase was more than offset by the improved data quality as 'hits' were generally defined as a percentage modulation. Several screens using novel targets had no positive controls and relied on the primary HTS to identify the first chemical modulator for that target. In some specific cases all compounds active above the assay 'noise' threshold were retested and the confirmed hits were further evaluated in functional assays.

Table 1 Successful prototype identification dependent on: sample diversity and number screened

Traditional screening	*High throughput screening*
Single tube	Array format 96-well
Large assay volume $\sim$1 ml	Small assay volume 50–100 μl
Compound used $\sim$5–10 mg	Compound used $\sim$1 μg
Assay components added singly	Assay components added simultaneously
Mechanical action 1 : 1	Mechanical action 1 : 96
Dry compounds—custom solution	Compound file in solution—DMSO
Assay slow and laborious	Assay fast and efficient ($\sim$1 min/step/96-well plate)
Screen 20–50 compounds/week/lab	Screen 1000–10 000 week/lab
Limited number and diversity screened	Unlimited number and diversity screened

Abbreviation: DMSO, dimethyl sulphoxide

Solvent and compound concentration

Of all the parameters subject to debate in the HTS arena, the identity and concentration of the analytes remains the most widely and hotly debated. Despite the diversity and range of physical properties of any large compound collection, a single universal solvent was desired so that all compounds could be solubilized quickly and efficiently and provided as solutions analogous to fermentation broths. Many of the established cocktails and various solvent choices were explored: DMF, DMSO, methanol, ethanol, mixtures with detergents and so on. DMSO solubilized the highest percentage of test compounds and is compatible with both *in vitro* targets and cells. It was empirically determined that most cell lines were unaffected by 0.1% DMSO while *in vitro* biochemical assays were more tolerant of 1–5% DMSO. There were two conflicting needs: maximum solubility, usually attained by using the most dilute solution, had to be balanced with the desire to have the highest concentration for screening. The latter need was driven by several philosophical considerations: (a) Many of the file compounds were unlikely to be potent at widely unrelated targets so we needed to identify all compounds from low to high potency to be successful, (b) Using flicked samples could change the nominal concentration fivefold and if only a single inhibition level was used to identify active compounds a number many would escape detection, (c) The stability and effective concentrations of compounds in DMSO was unknown so we wanted to address this from first principles, that is, screen at a high initial compound concentration so that even if 90% of the compound was unavailable the screening concentration would still be in the μM range. These considerations and constraints, together

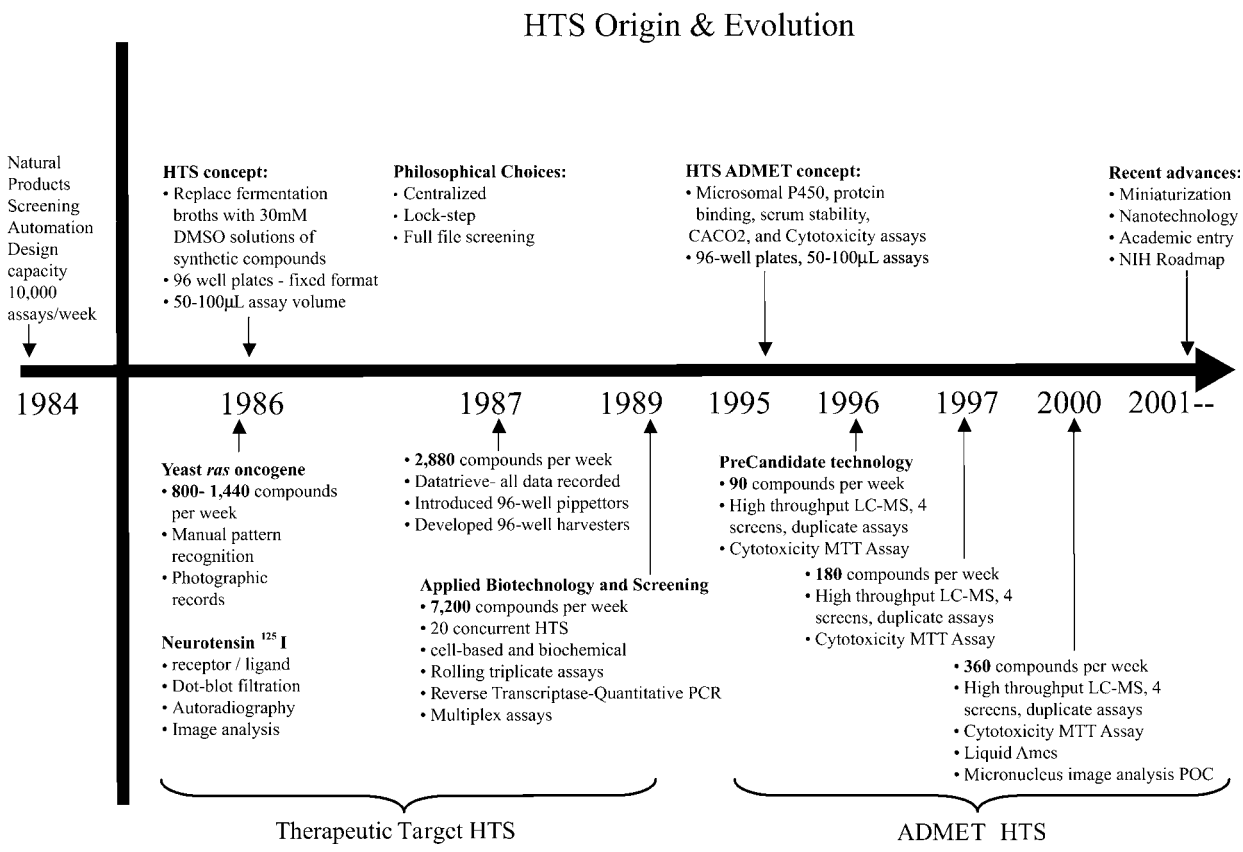

Figure 1 Chronological sequence of key decisions and developments pertaining to the origin and evolution of HTS.

with visual observation of solubilization, determined the most concentrated solution for 95% of compounds was approximately 30 mM, permitting screening at 30 μM with 0.1% DMSO.

Screening plate format

Once the 96-well plate format was established, the distribution of control wells was debated. Should each plate be independently controlled, how many wells were appropriate and which positions would be optimal? An alternative was to have 'control plates' instead and intersperse them every 5 or 10 plates throughout the assay. The range of control wells suggested by a survey numbered from 1- to 48-wells per plate, positioned from each corner of the plate to centrally within the plate. The final format was arbitrarily imposed with wells D 1, 2, 3 and D 7, 8, 9 blank for controls. This format was implemented as the standard and it has been successfully used at Pfizer since 1986.

Replicate screening

The healthy scepticism at the inception of HTS ensured that all screens were executed as traditional, independent triplicate assays to ensure valid data and sensitivity as there was considerable doubt that this approach would yield any 'hits'. It was imperative that the initial HTS assays were robust and designed to detect the desired activity with reasonable confidence. The initial tradeoff was to develop sufficient capacity to run large numbers of independent replicates in contrast to 'mixing' several compounds within each well. This approach offered two immediate advantages: (a) straightforward electronic data analysis to establish the benefits of replicates versus singlicate assays in the primary screen; (b) The initial capacity could instantly be increased if mixtures proved viable in later experiments. After several years of HTS in traditional triplicate assays we adopted a 'rolling' overlap model in 1989 to increase screening capacity as the DMSO library expanded. The model had three compounds per well with two compounds uniquely associated with 2 of the 3 wells so that hits were identified as 2/3 wells having the same modulation. This format also proved successful in all cell-based assays without general cytotoxic interference. The choice of pure compounds versus mixtures is still debated today after 20 years of HTS. The various positions are probably best understood in the context of the varying expectations of HTS. Primary HTS may be defined as merely detecting reproducible 'activity' for a compound. Success at achieving this modest goal has led to increased expectations and the demand for more subtle distinctions in activity to guide selection of the 'hits' to pursue. An extreme example is that of small thematic chemical libraries being screened to guide SAR with IC_{50} data. This is no longer HTS in philosophical terms, but rather focused screening in lead development. This latter concept has been adopted at several institutions where they have purified their entire collections to gain more precise data in primary HTS. This may be viewed as counter to the initial

concept as it adds significant cost and time to the process for a subset of active hits that represent $<5\%$ of the file.

Centralized or localized HTS with 'lock-step' or random access
Although we made our choice based on first principles this aspect of HTS has been continuously debated with the earliest published opinions on these two alternatives presented a decade ago (Hertzberg, 1996; Newbold, 1996). It still remains the subject of debate within many institutions and the arguments for and against are often coloured by personal preference. Centralized screening has the advantage of maximizing capital investments and synergy between screeners. Localized screening has higher capital costs and requires greater infrastructure to support several local systems; however, it has the perceived advantage of close proximity of HTS to the primary assay development team.

Centralized lock-step screening in its purest form is an approach in which a single stream of compounds is routinely provided to all HTS assays active at any given time. The stream of compounds is cyclical and consequently each assay will be screening various phases within the compound file dependent on its initiation. The major advantage is that compound distribution can be maximally leveraged since only a small fraction of the library is retrieved each week for all operational screens. Further efficiency is gained in that the production of multiple primary daughter plates is highly automatable. Extending this approach to confirmatory screening provides additional leverage as the same common set of mother plates can provide the unique subset of compounds requested for each individual screen. Therefore, screening 9000 compounds per week requires accessing only 100 source plates for all screens using a 96-well source format. This philosophy was successfully followed in the Applied Biotechnology and Screening Group, which operated $\sim$20 primary HTS assays on 7200 compounds each week, together with hit retests upto 3% for some screens. Retest compounds were supplied for all screens and required only 4 h using a single Probe-7 robot with a 24-plate deck.

Random screening advocates, whether centralized or localized, prefer the option of requesting any subset of the compound library. This approach uses directed screening by structural selection appropriate to the target in preference to empirical screening. The obvious inefficiencies associated with custom access in this approach are reasoned to be negated by the insights that are offered by more rational compound selection. Screening 9000 compounds per week using a 96-well source format requires accessing 100 source plates for each screen. Multiplying this number of source plates by the number of concurrent screens illustrates the magnitude of the task, for example 20 screens require 2000 source plates.

In vitro *biochemical versus cell-based screens*
Increased emphasis placed by the FDA on knowing the mechanism of action of any new chemical entity encouraged screening to pursue clearly defined biochemical targets. Cell-based screens were considered 'black-box' assays fraught with variability and the potential of a costly and lengthy unravelling of the mechanism of action of a hit. At the same time, however, R DNA was providing many intriguing reporter-gene constructs that were implicitly cell-based assays. So, notwithstanding that any number of cellular signals could trigger the reporter-gene response, a subset of cell-based assays was considered acceptable. An overarching advantage to using cell-based assays is that all the known and unknown signals/messengers that trigger the target of interest within the cell are identified in a single HTS passage, yielding a built-in screening capacity multiplier. An interesting substantiation of this principle is provided by a PDE IV hit (Duplantier *et al*., 1998) that was identified in a ligand-affinity biochemical assay. This was an acquired compound that was originally identified in an animal inflammation model, which is even a level higher in complexity than cell-based assays. HTS practitioners favoured predominantly one or the other approach.

During 1989–1994 a new group, Applied Biotechnology and Screening, developed and used reporter gene assays for low-density lipoprotein receptor (LDL-R) and many interleukins together with receptor binding, enzyme and ion channel screens. The efficiency of HTS was such that 1 person could screen 7200 compounds and confirm hits in 20 working hours.

A variation on the reporter gene approach was developed in 1991. This combined reverse transcriptase with quantitative-PCR (Q-PCR) for detecting modulators of the CSF family and other genes of interest, culminating in US and European patents (Banker *et al*., 1996). A major advantage was the ability to 'multiplex' targets within a single cell line. This translated into vastly increased efficiency, as each plate of cells required only a single addition of compound yet could identify hits for several targets by merely varying the target probe. A similar multiplex assay was implemented using a Hep2G cell line and both enzyme-linked immunosorbent assay and reporter-gene detection simultaneously. This approach permitted detection of modulators for ApoB, ApoA, fibrinogen and the insulin promoter all in a single cell-based assay. The ApoA was targeted towards elevating HDL without eliciting a concomitant increase in ApoB and LDL-R. The multiplex format permitted the simultaneous exploration of these targets and identified a number of ApoB inhibitors. One of these inhibitors was pursued using the nascent chemical high-speed analoging approach yielding nm inhibitors within 3 months and ultimately substantiating the cell-based screening principle when the lead hit was characterized as a microsomal triglyceride transfer protein inhibitor in a biochemical assay.

Full or subset file screening
An immediate philosophical choice with the advent of HTS was between screening all available compounds or a selected subset. Our stance was that since we were exposing new targets to file compounds synthesized for unrelated targets, full file screening would optimize HTS. This was based on principles of biological specificity as evidenced by enzyme specificity for stereoisomers and the distinction between active or inactive enantiomers. These are rather subtle structural changes that have dramatic impact on activity. It

seemed less than prudent to select a subset based on gross chemical structural diversity and expect to identify the widest range of active molecules. The counter philosophy, subset file screening, appears more intellectually elegant as it is based on knowledge of chemical structures and their diversity. Using computational expertise potentially ensures that maximal pharmacological space is explored with the fewest compounds, therefore at the lowest cost, an efficiency factor. Historically, this approach was also somewhat entrenched since, before the advent of HTS, it was the only way to explore the file diversity in a time and cost-effective manner. Major objections are: (a) subsetting is based on current knowledge of active molecules and the known active sites of known targets, thereby precluding novel molecules that might bind/interact at other sites on the target; (b) it ignores SAR data illustrating the dramatic effects of subtle structural modifications. HTS provides a cost-effective approach to examine the entire file rapidly. Our position was that complete file screening was optimal while subset screening is merely adequate. These two opposing philosophies were presented as point/counterpoint opinions a decade after the inception of HTS by Wikel and Higgs (1997) and Spencer (1997), and more recently by Valler and Green (2000), and today both approaches are practiced at different institutions. The explosion of compound library size resulting from combinatorial chemistry has revived the subset approach as the capacity of many standard HTS technologies has once again become limiting and introduced cost constraints. The advent and development of nanotechnology may well induce the pendulum to swing back again as cost per assay is decreased (Burbaum, 1998; Battersby and Trau, 2002). Historical data within Pfizer and other companies have substantiated the full file screening model when a single compound in the entire file has been found to be active for specific targets. One compelling example is that of the cholesterol ester transfer protein (CETP) inhibitor HTS that yielded a single hit with desirable secondary characteristics (torcetrapib) from a file of $\sim$350 000 compounds screened.

Practical implementation and technology development

When HTS was conceived there was limited instrumentation to support these processes and this led to the development of custom solutions, many of which have become industry standards. In 1987 the 96-well LKB β-Plate 1205 scintillation counter was introduced and it provided a major advance for high throughput receptor/ligand filter binding detection. However, it seemed ironic that the counter could process a single paper filter containing 96 samples while harvesting required manual processing of only eight samples simultaneously. We developed a Plexiglas prototype 96-well harvester with Tomtec and this prototype evolved into the highly successful commercial 96-well product, Harvester96.

An alternative detection device, Windowless Geiger counter, was developed by Digital Diagnostics Corporation in 1990. We were fortunate enough to work with them during early development and became a β-test site for the commercialized device by Packard – the Matrix96/9600. This device was essential for the HTS CETP inhibitor assay using ^{3}H-LDL and simple precipitation. Mylar sheets were spotted with 25 μl supernatant fluid and dried before counting. This permitted screening of the whole compound file, $>$350 000 compounds, quickly and cost effectively.

In 1986 all available liquid dispensers, manual or automated, were limited to 8- or 12-channel devices. A chance observation in a trade journal identified the Soken SigmaPet 96 as a novel 96-well liquid transfer device with a dynamic range of 5–500 μl. We started using these 96-well liquid dispensers in 1987 and this greatly facilitated screening capacity and speed. The original tips were made of TPX and were provided as 1000 tips/bag requiring hand racking. Tom Astle (Tomtec) suggested employing disabled workers in New Haven to rack tips and this was active for 18 months until a mould for polypropylene tips and racks was introduced for automated tip racking. These devices ensured rapid, accurate dispensing of assay reagents and compounds.

Data capture

At the inception of HTS there were no established systems with the capacity to record and capture HTS data. The first two HTS screens were a yeast ras oncogene screen and a neurotensin receptor antagonist screen. The former generated $9' \times 9'$ agar plates with yeast lawns and patterns of inhibition. Distinct patterns indicated the specific target inhibited that is, general cytotoxic, adenyl cyclase specific, yeast ras or human ras. These patterns were visually inspected and only the positive compounds were recorded in the database. A permanent photographic record of the plates was made. The neurotensin screen utilized a ^{125}I ligand and there were no detectors capable of reading the number of assays produced by the HTS. Drawing on R DNA and colony hybridization techniques we implemented an autoradiographic detection system using a dot-blot apparatus. Samples from the 96-well plate were transferred into a dot-blot apparatus with filter paper and washed. Sixteen 96-well filters were exposed on an X-ray film and produced black dots where the ^{125}I was bound. Any binding inhibitor resulted in intensity variations from black (no ligand displacement) to clear (complete ligand displacement) allowing easy visual assessment. A simple digitized image capture system was implemented and the data captured at the rate of 16×96 filters in $<$2 min. These data could then be loaded into the database and the advantages of structural trends captured by intensity variations. Another major advantage was the minimal concentration of ^{125}I required with the use of long autoradiographic exposures. This feature also ensured acceptance in Nagoya screening as Japan has stringent regulations regarding isotope use and quantity.

As noted above, the first HTS screen recorded only potentially positive compounds (by default all compounds screened were considered negative). The ability to digitize all screening data presented an unexpected obstacle. Assays were no longer merely positive or negative. It became necessary to quantitate in traditional formats that is, % inhibition, and so on. Challenges to achieving the latter included (a) personal computers were limited in both power

and user friendliness (b) the corporation's database capacity to load or retrieve data was not designed for the demands of HTS, (c) useful software for data manipulation and archiving was not available and (d) resource to accomplish all of the above was severely limited. The dedication of several individuals culminated in applications that provided computational and loading software, interfacing with the corporate database, Datatrieve from Digital Equipment Corporation (DEC), and daily support to users unfamiliar with the software and database. Before commercial packages became available in the early 1990s, a variety of data capture and analysis applications were developed by individuals in other companies that began screening.

HTS concept dissemination within the Pfizer discovery organization

Two new groups were created at Pfizer in 1987 to identify new prototype lead molecules, one in Groton (USA) and one in Sandwich (UK).

In Groton the New Leads Group was primarily focused on rapid chemical exploration of literature and patent disclosures. This was supplemented by rational selection of structurally related compounds in the compound file for screening against the target of interest as well as screening of limited subsets of the complete file. An external chemical acquisition programme was initiated to augment the Pfizer compound library and expand the pharmacological space, and fortuitously the first published small molecule, non-peptidic antagonist for substance P (Snider *et al*., 1991) was from one of these acquired collections. The group focused on enzyme and receptor ligand targets with pursuit of the related pharmacology. They became Pfizer's primary HTS group providing support for all Groton therapeutic zones.

In Sandwich the New Opportunities Research Group was comprised of chemistry and biology disciplines with a charter of providing a quick response to new information. Their strategy included a substantial screening component and they rapidly adopted the HTS approach implementing traditional *in vitro* biochemical assays. This group provided HTS support for all Sandwich therapeutic zones.

Ligand-affinity HTS

Development of a simple ^{3}H-cAMP assay for PDE IV enzymes offered another challenge: separating the cAMP from the AMP product. This was readily achieved using a boron ligand-affinity column in 96-well filter plates together with a custom 96-well elution device. The eluant fractions were assayed using the Matrix 96/9600 surface counters and Mylar sheets analogous to the CETP assay described previously. Once elution characteristics were established the assay became a 'batch' elution process as it was highly predictable.

Evolution of the HTS into ADMET

After 10 years of HTS it became apparent that, with all the HTS leads being produced, the screening bottleneck had moved into the ADMET zone. Once again it was conceptually simple to propose that HTS techniques could be applied to a number of key biological assays, namely, P450 enzymes, protein binding, general cytotoxicity using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, permeability using CACO2 cells and *in vitro* micronucleus enumeration. This proposal led to the creation of a new group, PreCandidate Technology, in 1995. A key difference was that assays required detection of individual chemical compounds in contrast to the normal common assay reporter. This hurdle was addressed through the development of tandem high throughput HPLC and mass spectrometry using the 96-well plate format with Gilson 215 robotic decks (Hiller *et al*., 1997). Innovative processing combinations, such as alternating between two, short, narrow bore HPLC columns, permitted sample separation on one column while the second column was regenerated. This was followed by automated electrospray MS using Scripts developed with Sciex, permitting detection of 90 unique compounds per week for all assays in 1996, with rapid escalation in the number of compounds to 360 by 1999. A unique 96-well micro-equilibrium device was created in 1997 to support high throughput protein binding (Banker *et al*., 2003a) as well as non-specific microsomal binding (Margolis and Obach, 2003) and patented (Banker *et al*., 2003b).

Early genotoxicity evaluation was implemented using a modified liquid-based Ames assay instead of the traditional spiral agar Ames assay. The modified assay was based on differential turbidity using a standard 96-well plate reader and required considerably less compound for testing. Another obligatory functional assay in the genotoxicity evaluation was the mouse micronucleus assay which was laborious and a true bottleneck. In December 1998, we evaluated commercial image analyzers and over the next several years new algorithms were devised and tested culminating in a patent application and recent publication (Xu *et al*., 2006).

Conclusion

The first formal HTS, presentations at national scientific meetings were within natural products sessions at annual conferences of the American Society for Microbiology and Society for Industrial Microbiology (SIM) starting in 1988. In 1993 SIM sponsored a dedicated workshop on Screening for Therapeutic Drugs Using Automated Assays in Toronto. There were several detailed presentations relating to data capture and analysis together with compound selection for subset screening. John Devlin convened a Data Management and Screening Technology meeting at Stanford Research Institute International in 1992 and the attendee response encouraged him to sponsor a second HTS conference, the Screening Forum Meeting, in conjunction with the Austrian Society of Experimental Biology in Vienna in December 1993 at which time the idea of forming a Society was raised. This idea was pursued at the second Screening Forum Meeting at Princeton in April 1994 and this led to the formation of the non-profit Society for Biomolecular Screening, incorporated in 1994 with an inaugural meeting in

Philadelphia in September 1994. The theme of HTS expansion from prototype hit identification to addressing bottlenecks in the ADMET arenas was accommodated with dedicated sessions at annual conferences and the formation of special interest groups. This aspect evolved and in 2005, 10 years after inception, the Society for Biomolecular Screening changed its name to Society for Biomolecular Sciences to more broadly reflect the divergence of HTS concepts within drug development.

In the 20 years since we implemented HTS it has become universal within the Pharmaceutical and Biotech industries as the major approach to identify novel prototype molecules for new targets. It has also spawned a billion dollar industry that supports the increasing demands for speed, capacity and cost effective screening of vast libraries of compounds. Many of the initial philosophical choices, such as full file screening versus computationally selected subsets, centralized versus distributed HTS, remain open and are supported on both sides by fierce adherents. Within Pfizer the key philosophical decisions and choices initially made, namely, DMSO, control format, centralized HTS, singlicate and mixed compounds sets, biochemical and cell based assays using the initial 30 mM concentration, still persist. Higher density formats, reduced volumes and lower screening concentrations, together with judicious use of full file screening versus subset screening dependent on the target type or family, have been introduced over time. Nanotechnology has added capacity, range and speed to the endeavor. Paralleling the natural HTS expansion into ADMET, HTS technology has also spilled over into many seemingly unrelated fields – genomics (Beggs and Long, 2002), high throughput crystallization, materials evaluation and environmental toxicity testing are some examples. The key to this versatility is the use of fixed formats generating tremendous leverage in mechanical access and manipulation, further evidenced by the progression to 384, 1536 and 3456 formats as straight forward efficiency levers. More recently HTS has been recognized by the academic community as providing an avenue for identifying chemical probes to investigate biological systems and the effects of target modulation. The NIH RoadMap initiative (http://nihroadmap.nih.gov/) is providing funding and opportunities to harness academic insights and expertise to fully capitalize the HTS concept for biological research.

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Conflict of interest

The authors state no conflict of interest. However, both authors were/are employed by Pfizer Global R&D.

References

Banker MJ, Clark TH, Williams JA (2003a). Development and validation of a 96-well equilibrium dialysis apparatus for measuring plasma protein binding. *Pharm Sci* **92**: 967–974.

Banker MJ, Davidson RE, Pereira DA (1996). Process for detecting specific mRNA and DNA in cells. Patents: EP 0 534640 B1 and US 5,643,730.

Banker MJ, Zuzel TJ, Williams JA (2003b). Micro-equilibrium dialysis vertically-loaded apparatus. Equivalent Patents: JP3400420(B2) and US 6,776,908.

Battersby BJ, Trau M (2002). Novel miniaturized systems in high-throughput screening. *Trends Biotechnol* **20**: 167–173.

Beggs M (2000). HTS –Where next? *Drug Discovery World Summer* **2000**: 25–30.

Beggs M, Long A (2002). High throughput genomics and drug discovery – parallel universes or a continuum? *Drug Discovery World Summer* **2002**: 75–80.

Burbaum JJ (1998). Miniaturization technologies in HTS: how fast, how small, how soon? *Drug Discov Today* **3**: 312–323.

Devlin JP (ed) (1997). *High Throughput Screening: the discovery of bioactive substances*. Marcel Dekker Inc: New York.

Duplantier AJ, Andresen CJ, Cheng JB, Cohan VL, Decker C, DiCapua FM *et al.* (1998). 7-Oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*] pyridines as Novel Inhibitors of Human Eosinophil Phosphodiesterase. *J Med Chem* **41**: 2268–2277.

Fox S, Wang H, Sopchak L, Khoury R (2001). Increasing the chances of lead discovery. *Drug Discovery World Spring* **2001**: 35–44.

Hertzberg R (1996). Centralized high throughput screening. *J Biomol Screen* **1**: 177–178.

Hiller DL, Zuzel TJ, Williams JA, Cole RO (1997). Rapid scanning technique for the determination of optimal tandem mass spectrometric conditions for quantitative analysis. *Rapid Commun Mass Spectrom* **11**: 593–597.

Janzen WP (ed) (2002). *High Throughput Screening: Methods and Protocols*. Humana Press Scientific and Medical Publishers, Totowa, NJ.

Kataoka T, Powers S, Cameron S, Fasano O, Goldfarb M, Broach J *et al.* (1985). Functional homology of mammalian and yeast RAS genes. *Cell* **40**: 19–26.

Margolis JM, Obach RS (2003). Impact of non-specific binding to microsomes and phospholipids on the inhibition of Cytochrome P4502D6: implications for relating *in vitro* inhibition data to *in vivo* drug interactions. *Drug Metab Dispos* **31**: 606–611.

Minor LK (ed) (2006). *Handbook of Assay Development in Drug Discovery*. CRC Press, Taylor and Francis: Boca Raton, FL.

Newbold RC (1996). An approach to distributed screening. *J Biomol Screen* **1**: 173–175.

Powers S, Kataoka T, Fasano O, Goldfarb M, Strathern J, Broach J *et al.* (1984). Genes in S. cerevisiae encoding proteins with domains homologous to the mammalian *ras* proteins. *Cell* **36**: 607–612.

Seethala R, Fernandes P (eds) (2001). *Handbook of Drug Screening*. Marcel Dekker Inc.: New York.

Sills MA (1998). Future considerations in HTS: the acute effect of chronic dilemmas. *Drug Discov Today* **3**: 304–322.
Snider RM, Constantine JW, Lowe III JA, Longo KP, Lebel WS, Woody HA *et al*. (1991). A potent nonpeptide antagonist of the substance P (NK_1) receptor. *Science* **251**: 435–437.
Spencer RW (1997). Diversity analysis in high throughput screening. *J Biomol Screen* **2**: 69–70.
Valler MJ, Green D (2000). Diversity screening versus focused screening in drug discovery. *Drug Discov Today* **5**: 286–293.
Wikel JH, Higgs RE (1997). Applications of molecular diversity analysis in high throughput screening. *J Biomol Screen* **2**: 65–67.
Xu JJ, Dunn MC, Smith AR (2006). Applications of cell-based imaging technologies in toxicity screening in drug discovery and development. *American Drug Discov* **11**: 20–26.