High throughput screening in academia

drug discovery initiatives at the University of Kansas

Academia has historically been involved in exploring the fundamental aspects of the disease targets and in developing tool molecules to better understand the genetic, biological and biochemical basis of new and novel therapeutic targets. Academia has now entered the high throughput screening arena which has long been the forte of the pharmaceutical industry. High throughput screening has not been the panacea for drug discovery as one was led to believe at first, but the prospects are improving. With the flow of top pharmaceutical drug discovery scientific talent into academia and the industrialisation of small molecule library synthesis, academia is poised to take new lead drug discovery to greater heights.

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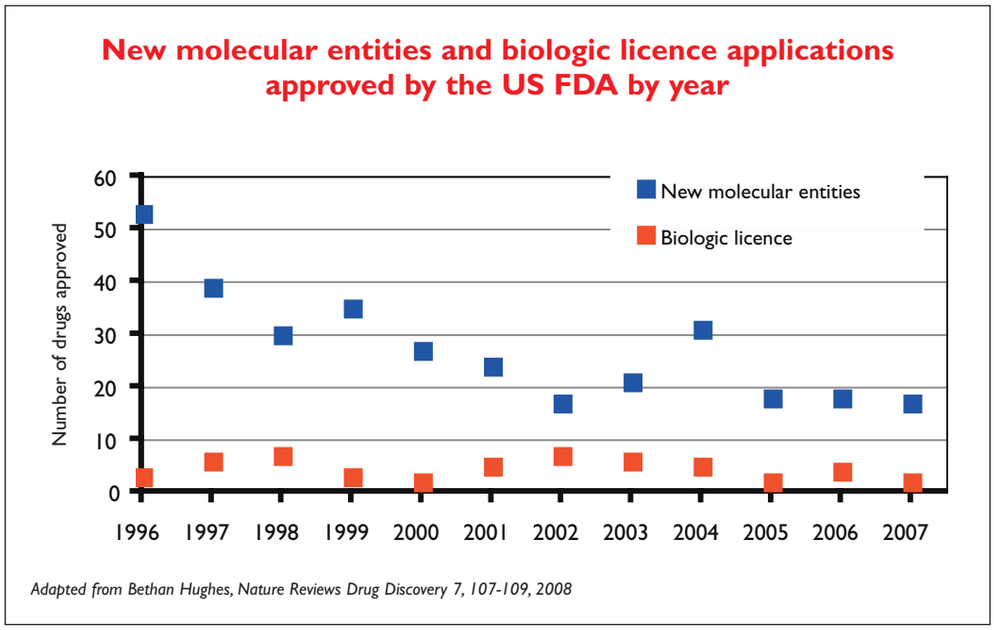
Academic research has contributed immensely in advancing the frontiers of science related to therapeutic areas. However, it has generally been the biotechnology and the pharmaceutical industries that contributed to the discovery and development of new drugs. The primary reason for this disparity has been the cost of the various facets of modern drug discovery. In recent years, high throughput screening (HTS) has been a major component in advancing new lead discovery research in Pharma. Academia and the smaller biotech firms simply could not participate in HTS efforts because of the high capital investment needed in setting up HTS laboratories. Similarly, Pharma neglected to address disease targets that are either financially too risky or scientifically impractical to implement. To bridge the gap in this disparity, academia is setting up HTS laboratories with funding from the appropriate government agencies to address the unmet critical needs by creating a new paradigm for drug discovery that integrates the best of big Pharma, Biotech and Academia. This has also become possible due to a change in the mindset of the academic researchers who long held the view that HTS is an anti-intellectual endeavour, but have since come to appreciate the strength of HTS in new lead discovery and in the development of molecular probes. The challenge that remains to be addressed by academia is the end goal of HTS. Is the goal just to find a 'tool' molecule or is it to discover and develop a 'drug'? What processes and collaborations have been set in place to transform a hit to a lead? With 'publish or perish' dogma that is prevalent in academia, protection of intellectual property rights is a formidable task even if one finds the proverbial 'needle in a haystack'. High throughput screening is a great concept and holds promise for academia, but to follow the pharmaceutical paradigm in transforming a screen hit to a lead, and eventually into a drug is easier said than done. The reality: academia is ready for it. The drug discovery and development initiatives at the University of Kansas (KU) are a prime example.

Figure 1



Changing landscape of drug discovery paradigm

The pharmaceutical industry, standing on the shoulders of academia, has been the driver of drug discovery and development by managing the knowledge for profit, with academia carrying research that one would call 'knowledge for knowledge's sake', and all in the name of academic freedom. It is now clear that the cost of research is escalating by leaps and bounds, while the number of new molecular entities as well as the number of drugs approved is at their lowest[1] (**Figure 1**). With the risk-averse posture of the pharmaceutical industry, however understandable, its main focus has been to go after financially rewarding therapeutic target areas and to shun areas with low return on investment – both in terms of sales as well as therapeutic success. On the other hand, academia has historically been involved in exploring the fundamental aspects of the disease targets and, of late, in developing the tool molecules to better understand the genetic, biological and biochemical basis of the therapeutic targets. The disease targets have largely been in the area of unmet medical needs including those that are rare and/or difficult to treat. Both the pharmaceutical industry as well as academia have now come to realise their respective strengths as well as weaknesses (**Figure 2**). What we now see evolving is the much needed collaborative spirit between these two diverse institutions in closing this risk-reward gap as exemplified by a number of industry-academia collaborative agreements that are being put in place (**Figure 3**). Pfizer has recently signed an ambitious multi-year, multi-million dollar, collaborative agreement with three University of California campuses involving a team of more than 150 scientists to carry on research of mutual interest. Likewise, Astra Zeneca has signed a multi-year collaboration with Columbia University to develop novel therapeutics for metabolic diseases, and Pfizer with the University of Pennsylvania-School of Medicine. GlaxoSmithKline entered into a five-year, $25 million collaboration, with the Harvard Stem Cell Institute. Pfizer has also entered into a collaboration agreement with several academic institutions – University of California-Santa Barbara, Caltech, the Massachusetts Institute of Technology and University of Massachusetts – and Entelos, a physiological modelling company, to re-examine the regulatory mechanisms of human energy metabolism, and hence expand the understanding diabetes and obesity pathobiology. In order to shorten the risk-reward gap, the pharmaceutical industry has again come to realise that the task at hand is too big to solve by any company on its own, and hence an unlikely, but much desired, camaraderie is evolving between competing pharma giants. Merck, Pfizer and Eli Lilly have now partnered together with PureTech Ventures to create a new company called Enlight Biosciences with initial funding totalling $39 million. Enlight Biosciences' charter is to develop 'breakthrough technologies that can fundamentally alter drug discovery and

development'. It is also heeding advice from Wall Street that successful companies will invest far more in creating a more holistic understanding of disease pathophysiology and epidemiology before embarking on development programmes (PricewaterhouseCooper, Pharma 2020).

The major challenges to the pharmaceutical industry in getting FDA approval for new drugs can be traced to failures in Phase I (safety) and Phase II (efficacy) clinical trials. Academia could offer its expertise in pathobiology to help the pharmaceutical industry in addressing the issues in these critical areas. With NIH's backing, academia has now advanced from hypothesis validation to the drug discovery arena, and is further augmented by the industrialisation of small molecule library synthesis and the greater capacities for synthesis of novel small molecules in acadmeic labs. Therapeutic target identification and validation has long been the strong suit of academia and its venture into HTS, and partnership with the pharmaceutical sector and the not-for-profit foundations creates the best opportunity the industry has ever seen in finding new and novel drugs to established, rare, as well as unproven, disease targets. The pharmaceutical sector has a better understanding regarding the fact that the ownership of intellectual property clearly resides within the academic institution, but that it has the rights to license the technology or has the first right of refusal if it does not intend to pursue commercialisation of the technology that came from funded research.

Bayh-Dole patent and trademark act

The new drug discovery paradigm in new lead discovery is based on complementation, not competition, between the pharmaceutical industry and academia. The genesis for this new paradigm takes its roots from the United States Congressional Bayh-Dole act of 1980 (Public Law 96-157). The passage of the act was primarily to induce '*economic development by promoting investment by the private sector in the commercialisation of federally funded research discoveries for the public good*'. Prior to

Figure 2

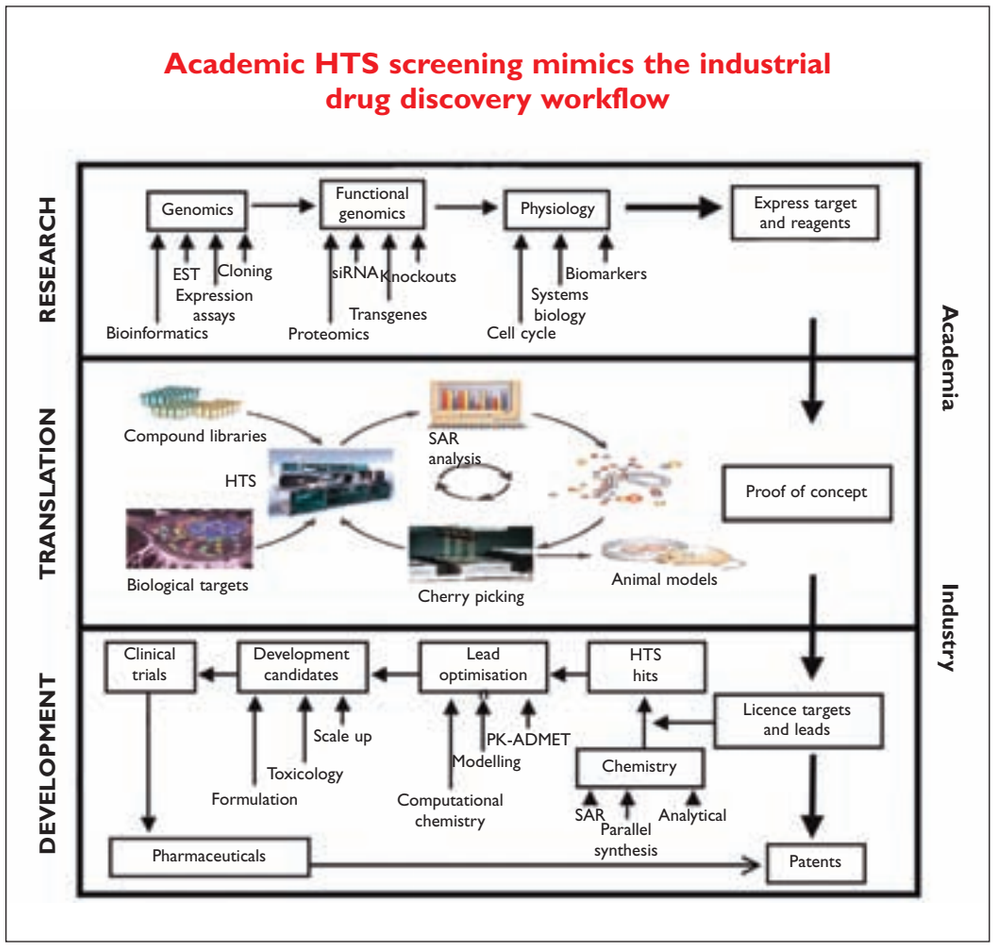


Figure 3: Industry-Academia partnerships

- Astra Zeneca signed a multi-year collaboration with Columbia University to develop novel therapeutics for metabolic diseases
- Pfizer signed a multi-year collaboration for $9.5 million to establish a team utilising 180 university scientists at the three UC campuses, collaborating on research of mutual interest
- Pfizer entered into a $15 million collaboration with the University of Pennsylvania School of Medicine in the areas of scientific research, clinical development and clinical care and policy
- Pfizer has also entered into a collaboration agreement with four major research universities – University of California, Santa Barbara Caltech, the Massachusetts Institute of Technology and University of Massachusetts – and Entelos, a physiological modelling company, to re-examine the regulatory mechanisms of human energy metabolism, and hence expand the understanding diabetes and obesity pathobiology

1980, United States Government-owned intellectual property (IP) from federally-sponsored research went in to public domain (publications, presentations, etc) without IP rights. It is understood that if one publishes without patent protection, the ability to convert the idea in to a commercial reality may have been destroyed prematurely and permanently. This prevented the industry from transforming the research ideas coming out of academia into practice. Further, there was also no easy mechanism in place for technology transfer from academia to the pharmaceutical industry. However, this changed after the passage of the Bayh-Dole Act which clearly mandated the IP to become the property of universities where the work was performed. This gave an incentive to the inventors to protect their intellectual property through patents. It has also been mandated that the universities have to report inventions to the Federal Government, providing an avenue to the US Government to either seek or maintain the right to patent research for which the universities themselves do not intend to patent. This has resulted in the creation of 'offices of technology transfer' within the academic institutions, and consequently a huge explosion in patent applications from federally-sponsored research. The Technology Transfer offices are now generally charged with the commercialisation of research results funded primarily by the federal government, essentially, for the welfare of the public. This involves the execution of confidentiality agreements, materials-transfer agreements, examining IP, licensing out technology and collaborative agreements with industry. To help facilitate this process, the Technology Transfer Office evaluates and guides IP disclosures and patenting decisions, management of patent prosecution and of existing licences (**Figures 4-6**). This has transformed the faculty into entrepreneurs in managing their research projects receiving support from federal or private sources. However, there is also a concern that the focus of research would be less basic and more applied, and that there would be an inherent loss of objectivity due to institutional conflicts of interest in equity management. While these are genuine concerns and of merit, it is clearly gaining momentum that a public-private partnership is a required necessity to advance drug discovery.

High throughput screening in academia

It is generally understood that most drug development expertise resides with Pharma, while the academia excels in basic biology related to the therapeutic targets, both conventional and non-traditional. Until recently, academia could not engage in drug discovery due to the lack of expertise in this field. With a stream of global cost-cutting campaigns resulting in huge job losses in the pharmaceutical sector and the ensuing availability of a pool of top talent in drug discovery, academia has now come to embark on strengthening its own drug discovery research programmes. The migration of the experienced investigators from the pharmaceutical sector to the 'greener pastures' in academia further improved this scenario.

The 'typical' academic screening centre operates alongside a department or school at the University with which they are affiliated. The KU-HTSL, for instance, relies on its affiliation with the University's Department of Medicinal Chemistry, as well as a relationship with the University of Kansas Medical Center. At its core, an academic HTS lab requires a variety of instrumentation, such as automated bulk liquid dispensers, liquid handling robotic platform and detection instruments, which may include an automated microscope for high-content cell-based analysis. Thanks to the pioneering efforts of industry HTS, many hurdles in automation design have been overcome, and screening instrumentation has become very affordable to the University lab.

Even with academic discounts, funding for both the creation and the maintenance of a screening lab is a major obstacle. Academic labs can acquire funding from various sources, such as startup funds, grants, donations and services. When an academic screening lab is being planned, it can often obtain start-up funds from its university, department, school, or even state, which have a vested interest in having an on-site screening core.

Grants can be pursued either directly by the screening lab principal investigator or through collaboration on a grant with another lab. Donations may also supplement the funding needs of the academic lab, since academic research usually pursues rare and difficult to treat human diseases. An academic lab may even seek payment for services rendered to cover personnel time and reagent consumption. Unique collaborations with the Department of Defense and the Army allow the government to team up with academic centres to target diseases soldiers face when stationed in other countries.

The heart of the HTSL is in its compound collection. The compound libraries are the 'crown jewels' of the pharmaceutical companies which include their own 'legacy' collections, and afford competitive advantage in their new lead discovery efforts. With the reduced cost of commercially available chemical screening libraries, augmented by the reduced cost of instrumentation, it has now become feasible for HTS laboratories to have their own collection of screening libraries. The compound libraries are typically composed of purchased library sets such as the LOPAC (Sigma), ChemBridge Corp (San Diego, CA), MicroSource (Gaylordsville, CT), ChemDiv, Inc (San Diego, CA) and Prestwick (Illkirch, France). Many academic labs have compound collections from commercial sources as well as analogue sets and combinatorial chemistry library sets developed by medicinal chemists within the university. Screening labs may also take advantage of increasingly affordable and commercially available small interfering RNA libraries (siRNA). siRNA libraries were originally available from only a few companies such as Ambion and Dharmacon, but now many other life sciences companies such as Invitrogen and Qiagen offer targeted libraries and whole-genome siRNA and short hairpin RNA (shRNA) libraries at affordable prices. These siRNA libraries are greatly useful in identifying genes involved in particular cellular pathways and processes. These were originally extensively used in worm and fruit fly-based systems, but now are being used to screen mammalian cells as well.

It is also worth noting that most of the recent graduates entering the pharmaceutical job market are inexperienced in drug discovery research, and have to go through rigorous on-the job training for an extended period of time to become competent discovery scientists. The 'internships' at HTS labs enable graduate, undergraduate and post-doctoral students to become aware of various aspects of drug discovery research including instrumentation, methodologies, chemical library management, target validation, assay development and data analysis.

Figure 4: Functions of technology transfer at KU

- **Assists** in making beneficial new products, processes and services available to industry and the general public
- **Encourages** the exchange of materials, information and personnel between the University and industry
- **Adds** to local and regional economic development by partnering with businesses and supporting start-up companies
- **Supports** the reputation of the region to attract more economic investment and development
- **Earns** licence income that may be used to fund new research, teaching programmes and research assistantships
- **Develops** opportunities for multidisciplinary students through internships and entrepreneurial coursework
- **Enhances** the reputation of the university to attract and retain the best researchers, faculty and students

Pharma versus Academia

The screening infrastructure and the workings of HTS differ between academia and industry, but all depend on instrumentation and compound collections for reagents and data reading and output. The smaller budget of academic centres imposes more stringent limitations on throughput and resources for academia than for industry. Thus the industry screening centres can afford multiple assay platforms, each designed for a specific assay/screen. Academic labs must do all of their own on one or two platforms and hence versatility is the key. Both the academic and industry screening centres can routinely screen 20,000-40,000 compounds per day. This level of medium to high throughput screening formats is sufficient and cost-effective for the academic centres. On the other hand, some pharmaceutical companies have large libraries of small molecules (>10^6) and are capable of performing ultra-high throughput screens of more than 100,000 compounds per day in high-density 1536-well formats.

The research projects are optimised for high-throughput screens in both industry and academia as both types of screening centres have intellectual and technical capabilities for optimising assays based on many approaches such as absorbance, scattering, fluorescence, luminescence, ELISAs, various solution phase binding technologies, enzymatic assays and many types of cell-based assays, including cell binding, toxicity and proliferation.

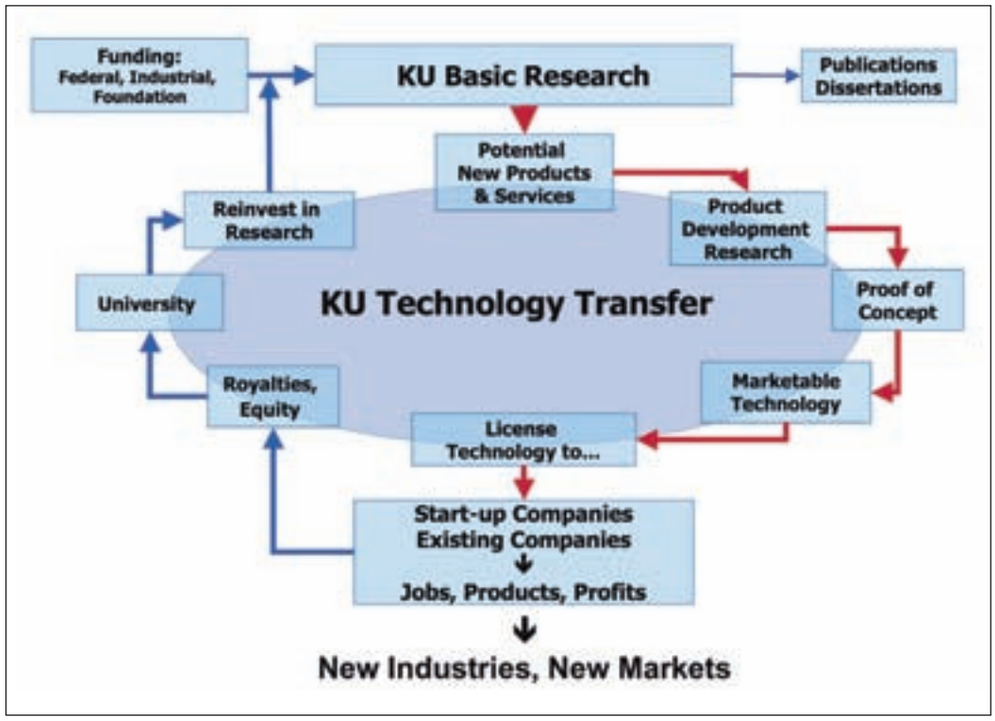


Figure 5

Pharma industry employs technicians that acquire specialised training in maintenance and execution of robotic systems. In contrast to this, limited manpower in academia extends handling of the robotic systems to all personnel including the students that intern in the laboratory. The screening system in academia is more flexible and smaller in scale. A small set of employees are trained to handle assay development, automation and data storage and handling.

Big Pharma has the capital and resources to set up state-of-the-art compound management and screening systems that are highly automated and integrated with liquid handlers, multimode plate readers, plate washers, CO_2 incubators, shakers and other screening platforms. Few academic centres possess integrated screening systems with a vast majority opting for stand-alone workstations to enable flexibility and cost-effectiveness.

Screening in an academic setting provides many advantages to the HTS lab, through networking, flexibility, idea sharing and freedom from the business model of industry. The academic set-up promotes networking with a wide variety of independent principal investigators, through university functions, vocational proximity and departmental seminars, allowing a mingling of researchers with varying backgrounds and research interests. This type of interdisciplinary interaction provides a fertile ground for novel ideas. Further, the inherent flexibility of the academic setting promotes open collaborations from the various fields of science, allowing for new avenues of research to be pursued in the screens. Assays or targets that may not be profitable enough for an industrial screening centre may still be very valuable to an academic screening lab, where publications can be based on discovery rather than shareholder interest. This allows screening targets to be focused on neglected diseases. Many diseases are neglected by pharmaceutical companies due to lack of profitability, since these diseases are too rare to affect enough people, or affect people who could not afford the potential drug that would be developed. This, to some extent, has been circumvented by Pharma which has cut costs by outsourcing R&D to contract organisations, academia and also via the takeover of Biotechs with promising leads in a wide spectrum of disease groups. In so far as drug discovery research in academia is concerned, academic screening does not even need to pursue a disease, for the discovery of a molecular inhibitor is relevant enough to biology to warrant a screening approach. This is one area that the industry relies on academia to provide, innovation and scientific ingenuity.

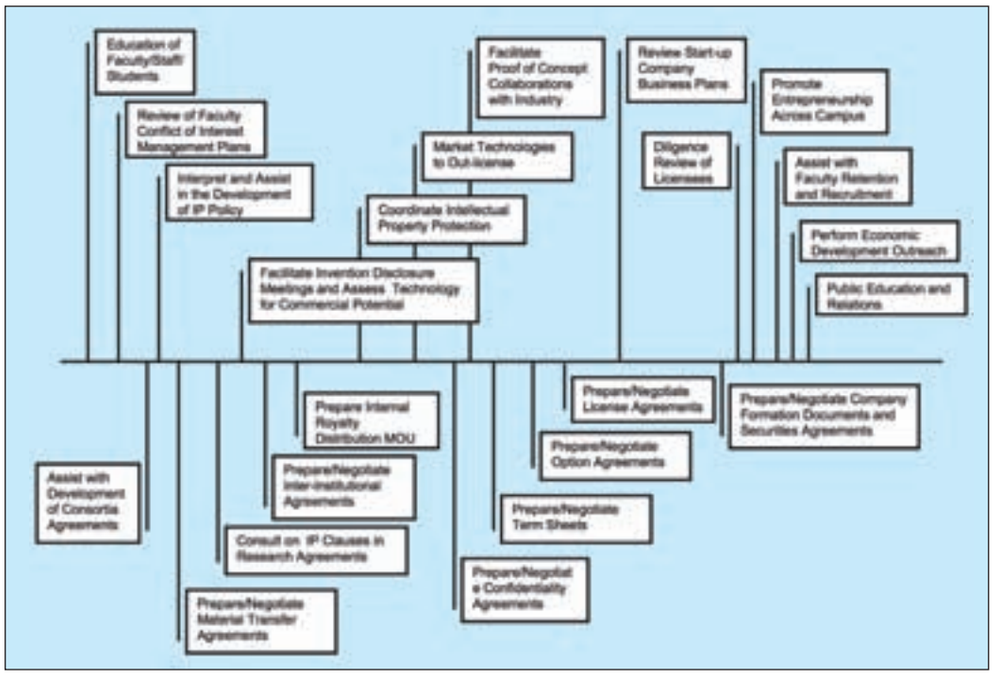
The benefits of being in an academic setting are offset by the disadvantages of trying to stay supported by funding and collaborative support. In

academia, there is a fierce competition for grants, which become harder to obtain as NIH funding shrinks. The tireless process of grant writing pulls time away from research, but encourages relevant experiments and efficient spending, to focus research dollars on important discoveries and targets. The collaborations of academic screening labs are vital to the drug discovery process, as screening hits need to be followed up, and analogue development relies on a strong connection to medicinal chemistry for a proper hit-to-lead strategy. Unfortunately, other project teams have their own research priorities, hindering and delaying necessary project collaborations. Further, most academic HTS centres do not even have access to medicinal chemists to optimise leads. Many academic HTS centres lack access to sufficient institutional resources and funding to carry hits forward to preclinical development. For an academic HTS lab to carry out successful drug discovery research, the infrastructure of the screening lab, biology, chemistry, structural biology, computational biology and pharmacology cores needs to be strong. As mentioned earlier, this is the area where the KU-HTSL distinguishes itself from the other screening centres in a very fundamental way by providing the 'value added' true drug discovery support.

The disadvantages of working in an industrial setting stem from the way in which executive decisions are made, and apply to all stages of the drug discovery process. Individuals or therapeutic areas are often given some discretionary funds to demonstrate that their therapeutic target is valid, and that it is drugable. However, instead of needing to convince a panel of scientists at a funding agency of the worthiness of their goal, researchers need to convince a committee of scientists and executives (triage process). The aim of the scientific review committees is to weigh the probable cost of a project and its likelihood of failure versus the likely profits of a new drug. Executive committees are usually more risk-averse than is government or private funding agencies, and creativity can be stifled. Instead, most drug companies end up pursuing the same well-validated drug targets and low-profit targets, such as diseases common in developing nations, are often neglected. While most grants provide funding for three to five years, a project in pharma can be terminated with little notice, and the project leader is forced to direct research efforts elsewhere.

The confidentiality with which research must be conducted provides another set of disadvantages to the industry scientist. While academic scientists have the freedom to consult any scientist/specialist, industry receives input from a Scientific Advisory Board, a panel of experts in the area of company's drug discovery research interests. The academic scientists are encouraged to publish their work as soon as possible, while the publications by their counterparts in industry in peer-reviewed journals are delayed, but filing of corresponding patent applications are encouraged.

Figure 6



NIH Roadmap Initiative

The US Government has long recognised the ability of universities to perform high quality screening services, and their utility in providing novel small-molecule chemical probes for basic research. In 2004, the National Institutes of Health (NIH) called upon the academic community, identifying 10 centres to screen a shared set of 200,000 compounds among them, to simultaneously identify probes against a variety of molecular targets for disease[2]. This network of screening centres was funded through the NIH Roadmap Initiative. This network of facilities, called the Molecular Library Screening Centers Network (MLSCN), provided a vast array of data and probes, and debased the negative stereotypes assigned to academic HTS[3]. Unfortunately, this was only a four-year pilot phase, and by mid-2008 seven of the initial 10 centres had been cut, as the Molecular Libraries Initiative (MLI) geared up for a production phase, focused on higher throughput efficiencies[4]. This left multiple academic screening labs searching for funding, joining other academic centres that are already desperate for grant support in a tumultuous US economy and shrinking NIH budget.

The squeeze of a decreasing NIH budget is felt by all academic researchers, and screening labs feel this pressure due to the high costs of consumables required when testing tens of thousands of chemicals. A primary cell-based screen of 100,000 compounds may cost upwards of $25,000, not including the hefty costs of assay development and optimisation. Follow up work and further experiments add to this cost, without guarantee of success. But to offset the decreasing government funding, screening labs can rely heavily on increasing relationships between academia and foundations, and the collaborative networks funded by public private partnerships and philanthropic organisations[5]. Further, university endowments can greatly strengthen core HTS services of an academic institution.

University of Kansas

The School of Pharmacy, the home for the Department of Medicinal Chemistry, is one of the nation's top schools in NIH funding. In a recent campus wide communiqué, the Chancellor of KU highlighted the $20.2 million National Institutes of Health award to Professor Jeff Aubé and his research team. The grant establishes a Specialized Chemistry Center at KU as part of a major initiative in the NIH National Roadmap to transform medical research during the next decade. Aubé and his team will work within the NIH Molecular Libraries Probe Production Centers Network to identify new molecules for fighting disease and advancing human health. Over the past 50 years, KU researchers formulated almost half of the cancer drugs that have come from the therapeutics branch of the National Cancer Institute.

KU is on the cusp of what academia is doing with HTS. It has an outstanding drug discovery and development programme in place and a strong and robust HTS component is part of the programme. The NIH funding through the COBRE (Center of Biomedical Research Excellence) grant to create the CCET (Center for Cancer Experimental Therapeutics) has been paramount in the establishment of the HTS Core Laboratory (HTSL). It was founded primarily to address the unmet critical medical needs by creating a new paradigm for drug discovery that integrates the best of Big Pharma, Biotech and Academia. The HTS effort at KU is based on the National Institute of Health's Roadmap programme. This helps ensure we are doing everything we can to ensure that a succinct and efficient programme is in place for discoveries made out of our labs. Furthermore, the KU-HTSL is unique in that we are completely integrated within the drug discovery and development programme across the matrix-campus. We have all the components in place to take a discovery from the bench all the way into the clinic for human clinical trials.

Table 1: Academic screening centres

SBS lists 39 'molecular screening centres'

- Loose definition of 'screening centre'
- Only 24 sites have >100,000 compounds
- These include biotechs, institutes and non-US sites

Limited number of actual academic HTS labs

- Only 16 US universities have >100,000 compounds
- Most lack medicinal chemistry departments
- Many are for in-house assays only

High throughput screening laboratory at KU

Academic screening centres range in size of labs, personnel and equipment, depending on the goals and funding of the particular lab (**Tables 1 and 2**). The Society for Biomolecular Sciences lists a total of 39 'molecular screening centres' worldwide. Among these centres, only 15 US universities have a large compound collection (100,000 or more) to facilitate a reasonable drug discovery effort and the use of most of these facilities are restrict-

ed to in-house investigators only. The KU-HTSL is unique in that it has a 100,000+ chemical library, a large number of legacy compounds synthesised in-house by the medicinal chemistry faculty members, a large collection of plant extract derived compounds and an open service policy. What sets the KU-HTSL apart is the value added support it provides with respect to target validation, assay development, purity check, structure confirmation, secondary/counter screens, medicinal chemistry support (hit explosion, structure-activity assessment, bio/chemoinformatics) and

Table 2: Academic high throughput screening labs

UNIVERSITY	CHEMICAL LIBRARY SIZE	DIRECTOR	WEBSITE
Broad Institute of MIT and Harvard	500,000	Michael Foley	www.broad.mit.edu/node/139
Columbia University	118,000	James Rothman	www.columbia.edu
Emory University	200,000	Ray Dingledine	www.pharm.emory.edu
Harvard Medical School	250,000	Caroline Shamu	iccb.med.harvard.edu
Johns Hopkins University	180,000	Min Li	www.hopkinschemcore.org
Rockerfeller University	46,000	J. Fraser Glickman	www.rockefeller.edu
Stanford University	130,000	David Solow-Cordero	htbc.stanford.edu
University of California	55,000	Scott Lokey	chemistry.ucsc.edu
University of Cincinnati	250,000	William L. Seibel	www.gri.uc.edu
University of Illinois	200,000	Carson Putt	www.scs.uiuc.edu/htsf
University of Kansas	110,000	Rathnam Chagaturu	www.hts.ku.edu
University of Michigan	56,000	Martha Larsen	www.umich.edu
University of Minnesota	5,000	Marc von Keitz	www.bti.umn.edu/htba
University of New Mexico	231,000	Larry Sklar	nmmlsc.health.unm.edu
University of Pennsylvania	218,000	Scott Diamond	www.seas.upenn.edu/~pcmd/hts
University of Pittsburgh	280,000	John Lazo	www.upddi.pitt.edu
University of Rochester	23,000	Alan V. Smrcka	www.urmc.rochester.edu/hts
University of Texas Southwestern Medical Center	200,000	Michael Roth	www.utsouthwestern.edu
University of Wisconsin Madison	105,000	F. Michael Hoffmann	www.hts.wisc.edu
Vanderbilt University	260,000	Charles David Weaver	www.vanderbilt.edu
Washington University	140,000	Jayne Marasa	mic.wustl.edu/Cores/HighThroughputCore
Yale University	30,000	Paul Fletcher	cgp.yale.edu/chemical/chem_info

intellectual property assessment. This distinguishes the KU-HTSL from the other screening centres in a fundamental way. The goal of the KU-HTSL is to make the new lead discovery effort affordable to institutional investigators, while training the next generation of drug discovery scientists.

The KU-HTSL is a shared resource of the university's Cancer Center headed by Dr Roy Jensen. The KU-HTS lab is located in a recently-completed wing amid a complex of eight-buildings containing most of the COBRE labs and other labs dedicated to biology, chemistry, and drug discovery (**Figure 7**). Within the COBRE-CCET framework, the KU-HTS lab's role is to provide assay development and screening services to academic, not-for profit and industry institutions. It has a fee-for-service cost structure, with in-house researchers paying the lowest cost, followed by other not-for profit, then for-profit institutions. Typical of academic screening centres, the KU-HTS lab was designed to carry out the widest possible spectrum of assays (**Table 3**). KU-HTS staff's knowledge base covers a broad range of cell and molecular biology, biochemistry, enzyme kinetics, and automation (**Figure 8**). The lab collaborates with experts within KU to fill in gaps such as medicinal chemistry, database management and high-content imaging. The KU-HTS lab also takes advantage of KU's large pool of talented undergraduates. Equipment, likewise, was chosen to maximise flexibility. High-throughput plate readers are available to read absorbance, luminescence, fluorescence intensity (including excitation and emission scans), fluorescence polarisation, time-resolved fluorescence and alpha screen. More moderate throughput equipment can do flow cytometry, high-content imaging and surface plasma resonance. A multi-modal inverted microscope with image analysis software is available to capture and analyse an image using any commercially available fluorescent probes. The chemical screening libraries were chosen to maximise structural diversity while favouring molecules with drug-like properties. The HTS lab also has access to a siRNA library, a natural compounds collection, and the medicinal chemistry's collection of compounds.

Integrated drug discovery initiatives at KU

The University of Kansas is recognised throughout the world as a top academic institution in the discovery, delivery and development of new and improved drug therapies. Capitalising on the strengths of the School of Pharmacy, efforts to establish an NCI-designated Comprehensive Cancer Center, and the wealth of drug development expertise residing in the region, KU has established a University-wide, fully integrated drug discovery, delivery and development organisation. The newly formed Office of Therapeutics, Discovery and Development (OTDD) helps drug discovery and delivery research which are then developed into innovative and improved products for the treatment, prevention and control of human and animal diseases. The OTDD has implemented pharmaceutical industry best practices to identify, advance and commercialise intellectual property, and has created a highly collaborative, entrepreneurial environment which attracts other academic institutions, industry and disease-focused non-profit organisations into partnership with the University. In keeping with the University's priorities, OTDD will place particular focus on cancer-related and neurological diseases. However, given the academic freedom of faculty, the OTDD will fully support faculty researchers in their research areas of interest should they fall outside of cancer and neuroscience.

Figure 7: Structural Biology Center at KU. Home of the high throughput screening laboratory

Scope of drug discovery, delivery and development at KU

Drug discovery activities at KU span target identification and validation through selection of optimised development candidates. Drug delivery focuses on the delivery of optimised development candidates to the site of drug action to maximise product efficacy and safety. Drug development encompasses the regulatory activities required to

advance optimised chemical lead candidates into clinical trials and commercialisation. The OTDD's goal is to provide world class drug discovery, delivery and development training to undergraduate, graduate and post-graduate students, and to create commercial opportunities for the University. The OTDD will make significant contributions to the growth of the state and national economy by supporting existing Kansas companies, attracting new companies to the region, supporting entrepreneurial efforts of existing faculty researchers, and attracting commercially focused eminent scholars and rising stars to Kansas universities.

These initiatives offer faculty a dynamic, highly interactive environment for collaborative research and professional development through access to thought leaders in drug discovery, delivery and development research; participation in projects, project teams, collaborative grant opportunities; and dedicated business development expertise to create opportunities to collaborate with industry, other academic institutions and disease focused foundations and societies. The OTDD will help facilitate the establishment of multi-disciplinary project teams supporting faculty projects through commercial outcomes with intellectual property management to secure, develop and commercialise faculty technologies and entrepreneurship training.

HTS lab start-up

With the challenges of funding and other limitations inherent to academic HTS labs, it is recognised that there is still great opportunity for changing the view of how and where quality screening takes place. From our perspective as an academic screening lab, we have several recommendations for the institutions contemplating starting an HTS lab at their institutions. A concrete budget plan needs to be developed to identify funding sources in addition to a pay-per-screen service. The starting point for start-up funds should be the parent organisation. It is in its best interest to have a screening lab, so it may be more than willing to provide the start-up funds.

Table 3: Typical HTS biochemical assays

ASSAY READOUT/TYPE	SUMMARY	EXAMPLE APPLICATION
Bioluminescence	Detection of bioluminescence by reporter gene assays	Luciferase detection
ECL	Electrochemiluminescence assay	ELISA based assays
Fluorescence – chromophores	Detection of fluorescent probes and antibodies binding to cellular components	Alexafluor detection
Fluorescence – kinetic	Rapid detection of fluorescence from calcium- or voltage-sensitive dyes	GPCR, ion channels
Fluorescence – proteins	Fluorescing protein detection from reporter gene assays and protein-protein interactions	GFP detection
FP	Fluorescence polarisation, ratiometric readout of polarised light alteration	Receptor-ligand interactions
FRET	Fluorescence resonance energy transfer	Kinase activity measurement
PCA	Protein complementation assay, detects interacting proteins	Luciferase, B-lactamase, GFP
TR-FRET	Time-resolved fluorescence resonance energy transfer	Kinase activity measurement

Developing collaborations and seeking inclusion on grants can provide much needed money for screening supplies.

When starting up an HTS lab, the 'wants' must be separated from the 'needs' with regards to equipment. The HTS industry has a variety of instrumentation in every price range and with various options for any given instrument type and from multiple vendors. Judicious selection is paramount to meet ones needs. For example, one need is a bulk liquid dispenser. Dispensers that do not use consumable tips can provide a cost-effective means of dispense cells and assay reagents with minimal cost. Tips can be very expensive, running up to $40 per box of 384 tips. However, tips are essential for automated liquid handlers, so one needs to keep in mind which automation platforms can use generic tips versus proprietary tips. Thankfully, many liquid handlers can be supplied with generic tips at a great saving from a number of life science instrumentation distributors. The signal detection platforms selected should be versatile enough for multiple applications, with the appropriate filter sets and fluorescent cubes for screening applications. In order to perform cell-based assays, cell culture incubators, sterile cell culture hoods and microscopes are needed in addition to the basic infrastructure of a research lab.

Many specialised instruments are impressive, but are not needed for weekly screening activities. Instruments that are rarely used or single-purpose should be avoided. Instruments that require expensive consumables or waste reagents through long priming paths or large void volumes in reagent reservoir bottles should be avoided as well. For example, a reagent reservoir with a bottom-feed may use 15ml less reagent than a reagent bottle with an inlet tube from above, which can be a small but very expensive difference. Some automation platforms that do not have swappable tip heads may limit you to a single function, while other automation platforms may be able to add that task with an additional tip head or accessory. Attention needs to be paid to total throughput and cost per well – having 1536 well capacity may appear to quadruple productivity relative to 384, but the optimisation required for 1536 capacity is considerably longer, and the more expensive instruments and tips required for accurate 1536 liquid handling may negate the proposed cost savings per well and assay point. Striking a balance between high maintenance versus use for instruments is of paramount importance. If a liquid handler requires more time for cleaning and flushing than for screening, or consumes more liquid in priming than in dispensing, it is best not purchased.

Highly versatile instruments are worth their weight in gold, such as large platform liquid handlers and automated microscopes. Alternatively, a

simple liquid handler may work very well as a workhorse, performing a frequent, simple pipetting task very well, with stability due to fewer moving parts. Since research dollars are tight in academia, a number of cost-saving measures could be put in place, such as tip usage and minimising void volumes for expensive reagents. This will also allow the researcher to spread the funding dollars to the farthest and help sustain the HTS lab as a core facility.

One of the bottlenecks created by the advent of microarray expression analysis was too much data, without a meaningful way of analysing it. To avoid this hurdle in high throughput screening, labs need to rely on information technology. The first step, data analysis, can often be done on the detection instrument or by exporting raw data, and analysing it with software programmes such as Spotfire, Attovision and MetaMorph. Some companies design their own proprietary software for their specific instrument, such as Cellomics' View software, designed for high-speed data analysis on-the-fly, processing data during the actual screening. Processing large sets of raw data requires trained researchers who can meticulously filter out key results from background noise, often with the help of automated macros and formulas, designed and implemented by the researcher. Data from high content cell-based screening, such as cell images captured with automated fluorescence microscopes, consume large amounts of server space, requiring a workflow for data storage and management. Large, in-house servers may store terrabytes of data produced from data-rich applications like high-content screening.

It is not unusual that many projects are going to be in the target validation or assay development stage. It is of paramount importance for HTS personnel to keep an open dialogue with the researchers on the various aspects of assay development and in developing an HTS-ready assay for HTS execution. An academic researcher is typically an expert on the biology or biochemistry of the therapeutic target, but not necessarily in executing his or her HTS project where precision is critical. The faculty most often does not have the budget to screen the entire chemical library and will most likely resort to do a partial screen, and thus limiting the chances of discovering a novel lead.

Figure 8: Standard HTS lab capabilities: KU as an example

HTS experience
- Assay development and optimisation
- Cell culture
- High throughput screening
- siRNA screening
- Data analysis/mining
- Hit-to-lead development

Assay formats
- End-point and kinetic
- Biochemical
- Cell-based
- High content analysis
- Biomarkers

Assay detection technologies
- Absorbance
- Luminescence
- Fluorescence intensity
- Fluorescence polarisation
- Time resolved fluorescence
- AlphaScreen
- Fuji SPR AP3000

Automated liquid handling
- Biomek FX workstation
- Tecan Genesis
- Thermo/Fisher Multidrops
- MDS Aquamax
- BioTek Precision
- BioTek Microplate washer

Signal detection platforms
- Perkin Elmer Envision
- Tecan Safire2
- MDS Spectramax Gemini XS
- MDS Spectramax Plus 384
- Perkin Elmer Victor 2
- Guava EasyCyte Plus

Cell imaging platforms
- Nikon Ti-S Microscope
- BD Pathway 855 HCS Miscellaneous
- Artel MVS Multichannel verification system

Miscellaneous
- Artel MVS Multichannel verification system

Summary

High throughput screening has not been the panacea for drug discovery as one was led to believe a decade ago. However, High Throughput Screening has found its niche in academia with research priorities in drug discovery endeavours. This is primarily due to a change in the mind-set of the academic researchers who long held the view that HTS is an anti-intellectual endeavour, but have since come to appreciate the strength of HTS in new lead discovery and in 'tool' molecule development. With the flow of top pharmaceutical drug discovery scientific talent into academia and the industrialisation of small molecule library synthesis, academia is poised to take new lead drug discovery to new heights. There is also a much needed collaborative spirit between Pharma and academia in closing the risk-reward gap as exemplified by a number of industry-academia collaborative agreements that are being put in place. The Technology Transfer offices of are now generally charged with guiding the researcher in IP disclosures, patenting decisions and commercialisation of research results. This has transformed the faculty into entrepreneurs in managing their inventions.

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Figure 9: Challenges imposed by the limited resources of academic HTS labs

- Assay development and validation is time-consuming and requires large resources of hardware, reagents and personnel
- Full library screens are cost prohibitive
- Data processing creates bottlenecks
- MTA, CDA and SA execution is cumbersome
- Hit processing methods are often undefined

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HTS laboratory's research priorities. Thanks are due to Dr Melvin Reichman (LCGC, Wynnewood, PA) and James Baxendale KU Center for Technology Commercialization) for their permission to use information included in some of the charts. KU-HTSL is a KU Cancer Center Shared Resource, and is funded in part by NIH/NCRR COBRE grant P20 RR015563. DDW

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