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The University of Kansas High-Throughput Screening Laboratory. Part I: meeting drug-discovery needs in the heartland of America with entrepreneurial flair

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Abstract

The University of Kansas High-Throughput Screening (KU HTS) core is a state-of-the-art drug-discovery facility with an entrepreneurial open-service policy, which provides centralized resources supporting public- and private-sector research initiatives. The KU HTS core applies pharmaceutical industry project-management principles in an academic setting by bringing together multidisciplinary teams to fill critical scientific and technology gaps, using an experienced team of industry-trained researchers and project managers. The KU HTS proactively engages in supporting grant applications for extramural funding, intellectual-property management and technology transfer. The KU HTS staff further provides educational opportunities for the KU faculty and students to learn cutting-edge technologies in drug-discovery platforms through seminars, workshops, internships and course teaching. This is the first instalment of a two-part contribution from the KU HTS laboratory.

Researchers in academia have traditionally been involved in understanding the fundamental aspects of disease biology and the associated biochemical targets, and in developing small molecules as tools against these biochemical targets to explore their therapeutic relevance. Historically, the pharmaceutical industry has not been very keen to address disease targets that are either financially too risky or scientifically impractical to implement. In recent years, academia has started entering into the high-throughput screening (HTS) arena, which has long been the realm of the pharmaceutical industry. Academic HTS laboratories address unmet critical medical needs and neglected diseases by creating a new paradigm for drug discovery that integrates the best of big pharmaceuticals, biotechnology and academia. The drug-discovery and development initiatives at the University of Kansas (KU) are a prime example. The migration of pharmaceutical scientific talent into academia, coupled with the commercialization of small-molecule library synthesis, enable the KU HTS core facility to take new lead-drug discovery programs to greater heights. Recent advances in technology, price maturation of the screening platforms and the availability of affordable chemical screening libraries have paved the way for this technology to become readily available to the academic sector.

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In order to have the HTS technology available to both public as well as private sector biomedical researchers in Kansas and beyond, the HTS laboratory was established in 2002 at KU with support from a National Institutes of Health Center of Biomedical Research Excellence Center for Experimental Therapeutics grant, the State of Kansas and the University; it is a shared resource for the University's Cancer Center and the Institute for Advancing Medical Innovation. The primary focus of the KU HTS core is to identify small molecules that can be used as probes of molecular and cellular pathways important in orphan and neglected diseases, and as lead chemical structures for future therapies. The KU HTS core is a central core facility in the larger initiative of drug discovery and development at KU. It is located in Lawrence, KS, a prime location in the growing Midwest animal health and life sciences corridor. This region of the Midwest is very involved in the life sciences industry, a home to thriving industrial biotechnology, bio-pharmaceuticals, agricultural technology, and literally hundreds of life-sciences companies. The region, state, and universities are pushing for competitive drug discovery, and HTS is essential to this drive.

What sets the KU HTS core apart is the fact that it is one of the only few academic institutions in the USA that has both a medicinal chemistry department and a HTS core; is completely integrated within the drug-discovery and development program across the matrix campus; provides support for target validation, assay development, secondary/counter screens, medicinal chemistry support, and intellectual-property assessment; and, most importantly, has an open-collaboration policy to support investigators' drug-discovery efforts in academia, biotechnology, not-for-profit, disease foundations and pharmaceuticals that do not necessarily have the infrastructure or time to execute HTS campaigns.

Project entrepreneurship at the core

The mission of the KU HTS is to expand the use of HTS and high-content screening for disease-related drug discovery at KU to identify small molecules that will ultimately result in drug treatments and therapies. The primary goal of the KU HTS is to execute high-throughput screens, while providing value-added support both before and after the actual screen. Before the screen, the laboratory offers consultation, assay development, basic research, technology-transfer assistance and grant application support. During the screen, the KU HTS core uses its core capacities including a large selection of screening instrumentation, as well as indirect services through its collaboration with other key University cores. Following the screen, the principal investigator (PI) is supported through hit-to-lead consultation, dose-response validation, secondary assays, medicinal chemistry support (cluster analysis, structure-activity relationships, hit explosion, structure-activity assessment, bio/chemoinformatics) and intellectual-property assessment. The strong support of the HTS staff by other cores and project managers provide a comprehensive drug-discovery solution to collaborators and clients, and distinguishes the KU HTS core from less involved screening centers. The goal of the KU HTS is to make new lead discovery effort affordable to investigators, while providing support at every step of the process.

Although localized at KU, we actively seek and engage in collaborations with investigators outside of Kansas, and do not limit ourselves to academia. One of the most valuable assets to the team is their experience in collaborating with academic, industry, and government researchers on assay development and HTS projects, which provides valuable insight into the dynamic relationship of the HTS core with assay providers and basic research labs. Currently, approximately 60% of our collaborators are from academia in Kansas, 25% are from other nonprofits, and 15% are from industry or biotechnology. These operations are guided internally by the director and project managers, as well as by the internal and external advisory boards. Project managers guide the progress of screening projects from the PI to the HTS staff to conclusion. Project management is carried out by dedicated managers

and by members of the HTS staff. Effective leadership is needed to identify critical go/no-go points and to guide the project development when needed. A target acceleration group has been useful in reviewing HTS project requests as to their technical merit, therapeutic relevance and HTS readiness. The KU Technology Transfer Office assists in intellectual-property assessment between the KU HTS core and the PI. This entrepreneurial infrastructure is critical to the KU HTS core because the relatively small size of the HTS core, as compared with pharmaceuticals, requires that each member carry a Jack-of-all-trades mentality. The skill sets of consulting, managing projects, assay design and development, biochemical and cell-based HTS and high-content screening, experimentation, screening, data analysis, interpretation and presentation, are critical to executing collaborative projects.

The KU HTS works collaboratively with other core facilities at KU, especially medicinal chemistry, microscopy and imaging, protein structure and expression/purification, and bioinformatics, to effectively address the needs of the investigators. KU HTS is a shared resource for both KU as well as KU Medical Center. Its operations are funded by Centers of Biomedical Research Excellence – Center For Cancer Experimental Therapeutics, KU Cancer Center, and the Institute for Advancing Medical Innovations (Figure 1). In addition to the internal KU service cores, the KU HTS has connections with local Kansas State University cores for other needs, such as specialized peptide synthesis, and collaborates when necessary with private industry contract research organizations (CROs) for other drug-discovery project assistance. For analoging hits and special compound synthesis, the KU HTS relies on the integrated medicinal chemistry core, located directly across the HTS core in the Structural Biology center.

The director of the HTS core, Rathnam Chaguturu, guides the HTS staff based on his more than 30 years of experience in new lead discovery and development, executing high-throughput screens, and managing hit-to-lead projects. He has led discovery research efforts focused on ion channel, receptor, enzyme and cell-based targets, and worked closely with academic faculty at various institutions in developing cell-based assays, carrying hit-to-lead development-related mechanistic studies, and executing targeted-discovery projects. Beyond his internal tasks of directing the HTS facility, he also actively participates externally, educating the public on HTS through seminars, training courses, conferences, and consults with new HTS laboratories in setting up and operation of their own HTS laboratories, both nationally and internationally.

In our mission to develop the next generation of scientists, we regularly train students at various levels of education, who participate in HTS related activities, providing a unique training opportunity to them. These ‘internships’ at the HTS labs enable graduate, undergraduate and postdoctoral students to become aware of various aspects of drug-discovery research including instrumentation, methodologies, chemical library management, target validation, assay development and data analysis.

Project workflow & management

The industry experience of the KU HTS director and project managers provide them with a useful framework for consultation with new collaborators (Figure 2). The KU HTS core provides a wide range of consultation services, including grant-application support well in advance of the actual laboratory work. Introductory consultations between the PI of the incoming project and the HTS core director, the project manager and the assigned HTS staff, clarify mutual expectations and maximize the PI's ability to use the collective resources of the HTS core. The HTS core staff assesses the technical aspects of the project, including

assay readiness, robustness of signal read-out, and funding availability. Where needed, alternate protocols and procedures are suggested for consideration by the PI.

Consultations with the Executive Director of the Technology Commercialization Office are facilitated to clarify the issues related to intellectual property. The PI is made aware that the data collected may be useful for purposes other than the immediate intent of the screen. Since all screens are run against the same collection of compounds, this provides a high level of annotation and information portal for all the compounds screened and the biological targets probed. Communication with the PI is continued throughout the project, during brief project updates and a final report compiling raw data, methodology and analyzed screening data with figures and conclusions, to assist in the publication of results. Screening data are stored and maintained in an in-house developed laboratory information management system, called K-Screen, for database management [1,101].

Costs for a project are either factored in ahead of time by inclusion of HTS in a grant, by collaboration agreement, or by fee-for-service when applicable. The calculation of costs follows a cost analysis, taking into account any extra reagent costs or special equipment needs. Due to the nonprofit nature of the KU HTS core and its function in a public university, costs for HTS are considerably lower than private HTS firms and CROs, with the added benefit of greater collaboration than private fee-for-service organizations and CROs. The HTS core usage costs are determined by the University's Financial Comptroller's office, with a three-tiered rate structure, priced according to the status of the collaborator in KU, other academia, not-for-profit or private industry. Despite the rate structure, all fees are flexible, and researchers with limited funding are encouraged to discuss with the HTS core director and the director of project management about their HTS needs, or to seek guidance on how to best proceed with a project.

Assay transfer to the HTS laboratory

Screens run through the National Institutes of Health Roadmap Molecular Libraries Probe Production Centers Network undergo rigorous application and validation hurdles to validate the proof-of-principle of the assay and its amenability to HTS. However, many academic labs with a valuable target do not have sufficient experience with HTS to miniaturize their assay, or even to apply it to a format suitable for screening a large compound library. The KU HTS core serves these researchers by offering multiple approaches to transfer an assay or target biology into a screen-able format, such as assay transfer and miniaturization, *de novo* assay development and alternative assay recommendations.

Most collaborators seek to transfer an assay to the HTS laboratory with as minimal changes as necessary. In these situations, if the assay is capable, the HTS researcher will miniaturize the assay as part of the assay transfer process, by titrating the reagents and components of the assay to optimize signal and maintain the metrics of the original assay as it is applied to a much smaller well format. The miniaturization of an immunofluorescence assay performed on slides, down to a 384-well microscopy plate, can yield the same data, but with fewer cells and less reagents, dramatically reducing costs while increasing throughput.

Some collaborators have a valuable target or biology, but use an assay method that cannot be easily transferred to the 384-well format without significant modifications to the methodology. In these cases, we either start from scratch or heavily modify the assay, and develop the assay from start to finish, finishing by testing known compounds to validate the assay with positive and negative controls.

Several recent collaborations have included assays that are technology-limited by the source laboratory. The instrument resources of the KU HTS laboratory allows for novel assay

methods using newer or rarer technologies such as AlphaScreen, label-free, acoustic and high-content analysis to provide new options for researchers that were not originally considered for their biological target. Simpler alternatives often appear during initial consultation with a new collaborator. For example, one recent PI who was using an absorbance-based method, but the KU HTS laboratory found the assay had significantly greater signal and robustness after conversion to a fluorescent approach.

Pilot data for grant application support

Providing grant application support often comes in the form of developing and optimizing an assay to an automated, HTS-ready format, then providing sufficient pilot screening data to the application. Many collaborators of the KU HTS laboratory need assistance in preparing pilot screening data for HTS assay grant proposals for screening through the Molecular Libraries Probe Production Centers Network [102]. A 1000–5000 compound pilot screen strengthens a grant proposal by demonstrating a proof-of-principle for the assay, as well as providing several compound hits. Since validation pilot screening is often carried out using compounds with known mechanisms of action, the type of screening hits can immediately validate the assay and target. In addition, the KU HTS staff also participate in writing R21 and R03 fast-track screening applications for collaborators.

Unifying complex multilaboratory collaborations through HTS core

The KU HTS core also serves as a middle ground, to pave the way for consolidating multi-investigator, multi-site projects under one roof. A recent collaboration of PIs used the KU HTS core as the central location to consolidate their different assays and different cell lines into one assay method, performed by one group, to unify their data. Prior to the collaboration, a team of KU investigators developed multiple analogs of the C-terminal Hsp90 inhibitor, novobiocin. The cytotoxicity of these analogs was tested in multiple labs, using various different assays and techniques, on cancer cells from a range of eight different tissue types. The team was interested in pursuing the best analogs for further analoging and testing, but the compounds could not be rank ordered sufficiently due to wide differences in methodologies and cell lines between the different investigators. Upon consultation with KU HTS, the group of investigators settled on a single assay, a homogenous, luciferase-based cell viability assay in a 384-well format. The KU HTS laboratory purchased all 19 American Type Culture Collection cell lines that were being used by the researchers, and created a liquid nitrogen storage cell bank of 475 cryovials, 25 vials per cell line, to maintain cell testing to be performed at the same passage number for each screen and optimization experiment. All 19 cell lines, two normal and 17 cancerous, were tested simultaneously at multiple concentrations, for four different time points of compound exposure, for 24 small-molecule compound analogs (Table 1). The results of these studies have helped determine the IC₅₀ values for of each compound in multiple cell lines in parallel, to provide insight into structure–activity relationships to guide compound prioritization toward the goal of therapeutic development of novel, potent, cancer-specific agents for clinical use [2]. The value of this consolidation was the minimization of variation due to differences in handling compounds, chemical stocks, labs, assays methodology, cell lines and data processing, and to prioritize compounds for further study. The throughput capacity of the KU HTS laboratory was essential in performing such a complex, multiparametric comparison assay.

Addressing scientific needs

High-throughput screening at KU provides many advantages to the academic faculty, 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 PIs, through university functions, vocational proximity, and departmental seminars, allowing a

collaboration of researchers with varying backgrounds and research interests. This type of interdisciplinary interaction provides fertile ground for novel ideas. Furthermore, the inherent flexibility of the KU HTS core 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 center may still be very valuable to an academic screening laboratory, where publications can be based on discovery rather than shareholder interest. This allows screening targets to be focused on neglected diseases. Insofar as drug-discovery research at KU is concerned, the screening effort 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 where the drug-discovery community at large (KU Lawrence, KU medical center, Kansas State University, and institutions from Central Region Institutional Development Award States) relies on KU to provide innovation and scientific ingenuity.

KU HTS in the wider context of drug discovery

The KU HTS core is a key shared resource in a comprehensive business plan for drug discovery at KU. This plan defined the implementation of a complete and robust drug-discovery process, through a 10-year business plan that includes recruitment of eminent scholars in medicinal chemistry; investment in R&D infrastructure such as HTS, target protein production, chemical library management, lead optimization, bioinformatics, combinatorial chemistry, process chemistry, preclinical proof-of-concept capabilities, drug-discovery training and education, seed funding, communication, and technology transfer; and construction of drug-discovery facilities.

Capitalizing on the strengths of the KU School of Pharmacy, the efforts to establish a National Cancer Institute-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 and development organization. The newly formed Institute for Advancing Medical Innovations helps drug discovery research activities, which are then developed into innovative and improved products for the treatment, prevention and control of human and animal diseases. The Institute for Advancing Medical Innovations has implemented pharmaceutical industry's best practices to identify, advance and commercialize intellectual property, and has created a highly collaborative, entrepreneurial environment that attracts other academic institutions, industry and disease-focused non-profit organizations into partnership with KU.

Future perspective

The KU HTS core is well situated in a supportive atmosphere to help collaborators not only with screening, but with all steps of the drug-discovery process (Figure 3) [3]. The university initiatives provide a dynamic, highly interactive environment for collaborative research and professional development through access to thought leaders in drug-discovery 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. Altogether, KU has an outstanding drug discovery and development program in place. A strong and robust HTS component is part of the program. The KU HTS core addresses unmet critical medical needs by providing a new paradigm for drug discovery that integrates the best of big pharmaceuticals, biotechnology and academia. The KU HTS core is empowered by its integration into the drug-discovery and development program across the campus, enabling discoveries from the bench to make their way into the clinic for human clinical trials.

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Figure 1. University of Kansas high-throughput screening core facility governance and financial management

Left: The KU HTS core collaborates with a variety of researchers in both the public and private sector. Top: The KU HTS is a shared resource of the KU RGS and the KUMC Cancer Center. Right: Funding is provided by NIH grants and private grants, along with support from the COBRE CCET, the KUMC Cancer Center, KTEC and KBA. Bottom: Governance is provided by both internal and external advisory boards, with individual projects managed by the collaborating principal investigator, along with the HTS director and project managers. Center: The KU HTS core collaborates with other KU cores to provide a greater range of services and capabilities.

COBRE-CCET: Centers of Biomedical Research Excellence – Center For Cancer Experimental Therapeutics; KBA: Kansas Bioscience Authority; KTEC: Kansas Technology Enterprise Corporation; KU: University of Kansas; KU HTS: University of Kansas high-throughput screening; KUMC: University of Kansas Medical Center; KU RGS: University of Kansas department of Research and Graduate Studies; PI: Principal investigator.

Figure 2. University of Kansas high-throughput screening project workflow

The standard collaboration between the University of Kansas HTS core and a public or private research principal investigator follows a set plan of managed steps, to ensure a smooth transition from the researcher's biology to a probe or lead molecule or series of molecules. Consultation includes assessment of the client's needs, plate format, assay requirements and readout, and often includes assistance with grant writing for future collaborations. Assays are either transferred, miniaturized, or developed in the HTS laboratory to ensure assays are robust enough for microplate format and automation. Optimization experiments are used to increase the assay signal while minimizing signal noise. The compound library screening is typically carried out with a small validation library of a few thousand compounds, followed by a larger library of 50,000–150,000 compounds. Primary screening data are analyzed as batches are screened, followed by dose–response testing and validation of primary screening hits. Cluster analysis is performed on validated hits and top screening compounds to identify structural similarity, structure–activity relationships, and patterns. Secondary assays are performed on validated compounds to determine specificity and selectivity to the target.

HTS: High-throughput screening.

Figure 3. University of Kansas high-throughput screening primary functions and services
 The University of Kansas high-throughput screening provides a range of services, from initial project consultation to medicinal chemistry support.
 HCS: High-content screening; HTS: High-throughput screening.

Table 1

University of Kansas high-throughput screening cell-line panel analog profiling[†].

Compound	Jurkat	HCT-116	HT-29	DRO	MDA-1986	JMAR	NPA	HT-1376	A549	HCC827	NCL-H226	MRC-5	NCH-H441	CoaV-3	OVCAR3	PC3MM2	LNCaP	MCF7	SK-BR-3	Average
KU-174	98	95	52	94	71	93	77	81	94	91	41	8	24	62	85	83	94	69	43	82
17-AAAG	81	91	80	82	77	88	91	74	76	53	39	74	12	86	34	47	66	61	35	75
KU-135	96	78	0	85	71	58	67	54	50	40	90	34	67	71	63	45	81	40	51	65
KU-131	96	96	0	74	66	91	82	70	52	44	77	18	48	63	45	36	35	47	24	57
KU-133	96	95	0	71	46	93	78	70	40	32	62	18	66	66	52	39	33	37	1	57
KU-179	90	83	0	86	71	83	63	40	62	44	42	20	29	0	52	43	53	38	0	48
KU-170	91	51	53	90	62	43	74	47	48	28	0	22	26	54	42	33	36	33	30	45
KU-113	80	84	48	61	44	19	39	54	82	42	11	0	9	46	11	78	57	81	7	45
KU-111	74	89	39	48	21	0	18	61	84	32	55	0	6	61	21	62	57	78	13	44
KU-171	90	45	0	84	71	51	60	45	40	33	52	8	22	42	20	32	40	36	12	41
KU-148	95	88	8	90	56	44	13	39	60	35	41	16	0	26	14	34	27	41	3	35
KU-139	93	63	11	58	81	67	52	33	62	35	36	8	15	31	22	34	24	27	0	35
KU-124	66	62	27	75	73	8	0	83	90	55	45	23	0	40	8	25	7	11	2	33
KU-130	82	45	1	41	53	12	25	39	36	18	0	13	40	58	17	33	25	23	1	29
KU-169	79	94	18	21	36	26	0	42	66	34	17	16	0	4	14	29	13	15	3	19
KU-152	49	61	12	14	31	56	4	14	28	39	25	12	0	20	7	23	13	28	3	17
KU-172	18	25	1	8	16	19	0	19	16	17	30	14	0	5	3	20	17	10	1	16
KU-115	29	16	15	0	18	11	15	33	35	17	40	14	0	40	0	21	0	0	2	16
KU-165	50	53	8	18	7	0	0	15	46	23	30	20	0	12	8	22	16	6	8	15
KU-128	23	93	15	0	22	21	0	24	66	14	6	5	0	18	5	14	3	2	2	14
KU-36	54	95	7	61	8	0	0	73	92	73	0	0	0	20	15	31	13	16	7	14
KU-173	22	42	0	10	0	21	0	24	0	14	46	11	0	0	4	21	52	23	0	11
KU-122	37	16	17	1	4	0	0	16	19	26	7	8	10	59	2	15	2	8	0	9
KU-146	67	78	4	39	0	0	6	37	68	25	27	3	0	2	12	38	0	26	0	9
KU-137	16	8	10	0	0	10	0	10	7	0	6	24	0	19	1	2	9	0	6	7
Average	67	66	17	48	40	37	31	44	53	35	33	16	15	36	22	34	31	30	10	-

[†]Numbers represent percent cytotoxicity. Compound analogs from a multilaboratory collaboration were compared by profiling cytotoxicity against a panel of normal and cancer cell lines. The compound screen included cells lines from over eight different tissue types. Certain tissue types were more sensitive to compounds, such as lymphocyte versus prostate. Within some cancer-tissue types, only specific cell lines were sensitive to selected inhibitor analogs, such as the two colon cell lines, where HCT-116 was significantly more sensitive to cell viability inhibition by the tested analogs.