Published in final edited form as:

Comb Chem High Throughput Screen. 2014 May ; 17(4): 298–318.

Chemical & RNAi screening at MSKCC: a collaborative platform to discover & repurpose drugs to fight disease

Bhavneet Bhinder, Christophe Antczak[†], David Shum, Constantin Radu, Jeni P. Mahida, Nancy Liu-Sullivan, Glorymar Ibáñez, Balajee Somalinga Raja, Paul A. Calder, and Hakim Djaballah^{*}

HTS Core Facility, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10065, USA

Abstract

Memorial Sloan-Kettering Cancer Center (MSKCC) has implemented the creation of a full service state-of-the-art High-throughput Screening Core Facility (HTSCF) equipped with modern robotics and custom-built screening data management resources to rapidly store and query chemical and RNAi screening data outputs. The mission of the facility is to provide oncology clinicians and researchers alike with access to cost-effective HTS solutions for both chemical and RNAi screening, with an ultimate goal of novel target identification and drug discovery. HTSCF was established in 2003 to support the institution's commitment to growth in molecular pharmacology and in the realm of therapeutic agents to fight chronic diseases such as cancer. This endeavor required broad range of expertise in technology development to establish robust and innovative assays, large collections of diverse chemical and RNAi duplexes to probe specific cellular events, sophisticated compound and data handling capabilities, and a profound knowledge in assay development, hit validation, and characterization. Our goal has been to strive for constant innovation, and we strongly believe in shifting the paradigm from traditional drug discovery towards translational research now, making allowance for unmet clinical needs in patients. Our efforts towards repurposing FDA-approved drugs fructified when digoxin, identified through primary HTS, was administered in the clinic for treatment of stage Vb retinoblastoma. In summary, the overall aim of our facility is to identify novel chemical probes, to study cellular processes relevant to investigator's research interest in chemical biology and functional genomics, and to be instrumental in accelerating the process of drug discovery in academia.

Keywords

HTS; HCS; RNAi; siRNA; shRNA; miRNA; automation; robotics; small molecule; chemical; robotics; cell-based assay; target-based assay; screen data analysis; drug discovery

^{*} Author for correspondence: Hakim Djaballah, PhD, Director, HTS Core Facility, MSKCC, New York, USA; Tel: (646) 888-2198; djaballah@mskcc.org.

[†] Current address: Novartis Institutes for Biomedical Research, Center for Proteomic Chemistry, 250 Massachusetts Avenue, Cambridge, MA 02139, USA

DISCLOSURE STATEMENT

The authors declare no competing financial interests.

INTRODUCTION

The HTS initiative first started with the Pharma giants and gradually made its way into academic institutions, however very few had the resources or the commitment from the senior management for successful set-up and implementation of such an enterprise. MSKCC recognized the need to have a state-of-the-art facility that would advance scientific research to probe potential drug candidates in a fight against debilitating diseases like cancer. HTSCF was established in 2003; with its conception in a small room in the Rockefeller Research Laboratories building, hundreds of thousands of tiny chemistry experiments were run simultaneously, each holding a promise to yield potential leads in the field of oncology. HTSCF gradually became an engine for identifying potential anticancer drugs and a resource to help investigators at MSKCC to meet their research goals and accelerate drug discovery. In 2006, HTSCF was moved to the newly constructed Mortimer B. Zuckerman Research Center, a building referred to as a paradigm of urban laboratory; and HTSCF was allocated laboratory space on the 19th floor of this 558,000 sq ft 23-story building; the increased bench space provided a valuable incentive to expand the automation capabilities (Fig 1A). In 2010, MSKCC yet again showed its commitment to the HTSCF when the screening efforts were expanded from HTS to high content screening (HCS). In order to automate the stand alone HCS microscopes, namely an alpha IN Cell Analyzer 3000 (INCA3000) and the IN Cell Analyzer 2000 (INCA2000) MSKCC invested into a second automated robotic system referred to as Hestia, which harbored the only fully automated alpha INCA3000 unit in the World.

A decade later, HTSCF features a vast collection of chemical compounds, RNA interference (RNAi) duplexes with up to genome-wide coverage, cutting-edge robotics and instrumentation, in-house data analysis capabilities, custom-built data management resources and experienced staff that provides investigators with access to rapid, cost-effective HTS and HCS solutions. In contrast to most academic screening core facilities, the HTSCF provides a full service to MSKCC investigators that covers assay development, optimization and validation, assay miniaturization and industrialization for screening, image and raw data analyses, and confirmation of selected hits (Fig 1B). Since its inception, HTSCF has collaborated with internal and external research groups to successfully completed multiple chemical screens, small interfering RNA (siRNA) duplex screens, short hairpin (shRNA) hairpin screens and microRNA (miRNA) mimics screen with a broad coverage in the areas of oncology as well as neglected tropical diseases such as dengue, utilizing innovative assays and providing continued assistance in hit validation and characterization. Successful collaborations and independent in-house research efforts yielded in publications with significant contributions to the scientific community. The experience of our staff plays an important role in producing robust and reproducible data procured from unbiased data analysis practices. To maintain high professional standards and a safe work environment, we have adopted a zero tolerance policy at HTSCF in terms of bio-safety and adherence to set guidelines for laboratory operations. In this report, our infrastructure, resources, project workflows and extended capabilities are described.

HTSCF management structure

MSKCC is committed to a strong centralized management of all core facilities at the Center to ensure that optimal services are provided to support the Center's research mission. For this purpose, the Office of Core Facility Operations provides oversight to all management aspects of the cores at MSKCC; reviews the infrastructure needs of each facility, including space, equipment, staffing levels, and monitoring budget and cost effectiveness of the operations (Fig 2). In addition, an Oversight Committee is also designated which meets at least once a year to review, discuss, and make recommendations for the overall prioritization of HTS projects, directions, capital equipment, and strategic planning. The HTSCF director is responsible for the administration and the day-to-day running of the facility; to provide consultations and guidance in design of experiments, in analysis and interpretation of experimental data.

Members of HTSCF are divided into three discrete teams namely, assay development and screening, compound management and automation, and data management. The three teams work in close collaboration and under the directives from the director of the HTSCF. All the HTSCF members meet once every week to provide regular progress updates on all current projects and to discuss forthcoming steps. In addition, the members of the core are required to be well versed with the recent publications in their field of expertise so as to stay at par with novel technologies relevant to the mission of the HTSCF, fostering analytical and critical skills. Our belief is also to provide exposure and create growth avenues for bright young minds with a keen interest in scientific research, and therefore we readily provide opportunities to students and postdoctoral research fellows to gain experience and training in HTS and drug discovery in general.

HTSCF resources & capabilities

A) Diverse chemical and RNAi library collection

Chemical library—The HTSCF has assembled a diverse library of approximately 400,000 chemicals. The chemical space of the library is made up of synthetic chemicals, natural products from plants, micro-organisms, fungi, and deep sea algae, selected on the basis of dissimilarity. The collection was assembled from several established vendors with some compounds selected according to Lipinski rules for drug-likeness and additional criteria to eliminate reactive and otherwise undesirable functional groups/moieties. Our compound collection is obtained from AnalytiCon Discovery (Potsdam, Germany), ChemBridge (CA, USA), MicroSource (CT, USA), ChemDiv, (CA, USA), NCI (DC, USA), Prestwick Chemical (Illkirch, France), Specs (Delft, The Netherlands), SeqChem (Pangbourne, UK), Sigma-Aldrich (MO, USA), Selleck Chemicals, (TX, USA), Sarawak Biodiversity Centre (Sarawak, Malaysia), Tocris Bioscience (Bristol, UK), and Magellan BioScience (FL, USA), the David Gin collection, as well as internal suppliers (Table 1).

Chemical library storage—Chemical libraries are obtained from the vendors in a 96- or 384-well microtiter plates, either in a liquid form or as dry powder; the latter is dissolved in 100% DMSO (v/v) and, if in 96-well microtiter plates, then the plates are compressed into 384-well polypropylene plates. The stock of our libraries are formatted and stored in a

polypropylene 384-well source plates (SPLs) with a final compound concentration of 10mM. Each SPL is further diluted into individual daughter plates (DPLs) at 1mM concentration dissolved in 100% DMSO (v/v). Each DPL is diluted even further into intermediate plates (IPLs) at 100 μ M concentration in 10% DMSO (v/v). The SPLs and DPLs are stored in automated Biophile freezers at -20°C constantly purged with nitrogen. To ensure minimal freeze-thaw cycles of the SPLs and DPLs, the IPLs will be used from this point on to make the assay plates (APLs) per project at 10 μ M final screening concentration in 1% DMSO (v/v). By rule of thumb, the shelf-life for the APLs is about 6 to 12 months; beyond which the APLs should be discarded. In addition, great care is taken with regards to compound plating and liquid handling; only fresh sterile disposable tips are used for liquid and compound transfer. All the steps detailed here are undertaken to ensure that the integrity of our compounds is not compromised at any stage of the HTS process.

RNAi library—The MSKCC siRNA library was the first RNAi library acquired by HTSCF; the MSKCC collection of siRNA duplexes were synthesized using our custom algorithm and cover approximately 7,000 human genes; and with an average coverage of 3 siRNA duplexes per gene. With the completed expansion, we purchased the Ambion® Silencer® Select V4.0 Library from Ambion® Life Technologies (CA, USA), targeting 22,000 human genes with an average coverage of 3 siRNA duplexes per gene. This siRNA duplex collection is chemically modified to help attenuate off target effects (OTEs). In 2009, we further expanding our capabilities to undertake shRNA hairpin screens by purchasing the MISSION® shRNA hairpin library (The RNAi Consortium, the TRC1 library) from Sigma-Aldrich (MO, USA) consists of 159,000 shRNA constructs targeting the human and mouse genome. The TRC1 Library covers approx 16,000 genes for both human and mouse with an average coverage of 5 shRNA hairpins per gene. The TRC1 Library is pre-plated as lentiviral particles and ready for screening. We also acquired the glycerol stocks for all the hairpins represented in the TRC1 library and an additional collection from the TRC1.5 library. For 4 years, we had efficiently supplied these glycerol stocks to the investigators at MSKCC at a discounted price to assist in their research endeavors, after which these services were divested to the newly created RNAi Core Facility concentrating on the RNAi technology developed at Cold Spring Harbor Labs. Our current RNAi collection has significantly expanded to include custom built, focused and up to genome-wide collections from various vendors (Table 2).

B) Scientific instrumentation & detection technologies

For low content cell-based assays, biochemical based assays and affinity based assays, the HTSCF is equipped with the following detection instrumentation: two Perkin Elmer VICTOR3 V™ Multi label counters, including fluorescence intensity, fluorescence polarization, time-resolved fluorescence, absorbance (UV/VIS) and luminescence technologies. These counters also include red sensitive PMT (for better TR-FRET performance); both counters are integrated on a robotic platform [1] but can also be used as standalone during assay development and can read 96-, 384-, and 1536-well microtiter plates. One Wallac Microbeta® TriLux counter with 12 detectors and includes liquid scintillation counting and luminescence which is suitable for assays using ^3H , ^{14}C , ^{32}P , ^{33}P , ^{35}S , ^{51}Cr , ^{125}I and glow-type luminescence labels as well as for ScintiPlate,

FlashPlate and scintillation proximity assays. It counts filters and tubes in addition to microtiter plates. Two Molecular Devices SpectraMax® Plus384 spectrophotometers for absorbance scanning and real time kinetic measurements such as coupled assays for kinases; both units are integrated on the robotic platform [1] and can also be used as stand-alone. One BMG laser based microplate nephelometer, the Nephelostar, for measuring light scattering in colored or turbid solutions and used for measuring compound solubility limits in aqueous solutions. One GE Healthcare LEADseeker™ Multimodality Imaging System, a CCD-based optical imaging instrument, which efficiently detects and quantifies light from a range of radiometric, luminescence, and fluorescence technologies enabling whole plate readout at once. This system can image 96-, 384-, and 1536-well microtiter plates. This variety of detection instrumentation enables us to carry out screening in 96-, 384- or 1536-well microtiter plates using any of the multiple and diverse detection and analysis technologies (Table 3).

C) Imaging technologies

For high-content cell-based assays, the HTSCF is equipped with three automated microscopes, integrated to our robotic platforms. One GE Healthcare INCA1000, an automated and flexible epifluorescence microscope system capable of image acquisition and analysis of fixed or live cell-based assays from 96- and 384-well plates at speeds of up to 2,000 images per hour. The instrument is capable of flexible liquid handling, and is fully integrated with one of our robotic platforms. For live assays, cell integrity is assured with continuous control and reporting of the temperature during image acquisition. One GE Healthcare INCA2000, a wide-field automated epifluorescence microscope equipped with a large-chip CCD camera (2048 × 2048 pixels) that allows whole well imaging in 384-well format. Whole well imaging reduces acquisition time hence increasing throughput, and also enables to image multi-cellular formations, important for example to study cell transformation and spheroids, relevant to current cancer research. One GE Healthcare INCA3000, the only fully automated alpha unit in the world, is a laser scanning confocal microscope capable of high throughput sub-cellular screening. It contains two solid laser light sources (Argon and Helium/Neon) with three highly sensitive onboard 12-bit CCD cameras. It can image fixed or live cells in 96- or 384-well plates at speeds of 1,200-3,600 wells per hour depending on image resolution. This speed of operation can be achieved as a result of several factors: 1) a plate surface sensing autofocus mechanism minimizes focus time to less than 200 ms/well, 2) the system is capable of simultaneous image acquisition in 3 color channels using laser-based excitation coupled with three independent low noise CCD cameras, and 3) unlike conventional confocal microscopes that use slow moving point scanning to form images, the INCA3000 uses a very fast fixed line scanning system. In addition to providing increased speed, line scanning optics are devoid of moving parts, and can accommodate a larger field of view, with more cells in a given image (typically 500 cells/image). Images acquired by the instrument are analyzed in real-time by object recognition algorithms that can extract fluorescent marker data from each and every cell in a given image. Cell by cell quantification of the intensity, size, number and relative localization of multiple fluorescent markers can be customized to any cell-based assay by modifying the many user-definable parameters of these algorithms. Because a single image

contains so much information in the form of individually analyzable cells, statistical analysis of cellular responses to modulators is embedded within the measurement (Table 3).

D) Integrated robotics platforms

The HTSCF employs three distinct integrated robotic platforms: *Xanthus*, *Isis*, and *Hestia* [1], to rapidly and efficiently screen our chemical, siRNA duplex and shRNA hairpin library collections covering the broad range of detection technologies previously described (Fig 3).

The Xanthus robotic platform—The first linear robotic platform at HTSCF was built in 2003 using a CRS F3 robotic arm (Thermo Fisher Scientific, MA) on a five-meter linear track surrounded by several peripherals [1]. The instruments were strategically positioned on the track therefore enabling their automated usage by the robotic arm, while also keeping them accessible for standalone use or service and maintenance purposes. The instruments surrounding included LEADseeker, VICTOR3 V, INCA1000, four high humidity Cytomats CO₂ incubators at a capacity of 1,389 plates and two room temperature incubators at a capacity of 392 plates; together is enabling automated live cell-based high content assays. The platform also housed two BioTek Elx405 plate washers, one ABgene 300 plate sealer, one LidPark lidding station capable of unlidding/lidding up to seven sample plates, three MultiDrop and two FlexDrop automated liquid dispensers.

The Isis go anywhere platform—In 2010, we purchased an Orbitor RS Microplate Mover (Thermo Fisher Scientific, MA) with nine stacks and a capacity of 320 384-well format plates. The high-speed Orbitor provided us with operational flexibility and could be integrated to any instrument in a small footprint.

The Hestia robotic platform—This robotic platform was installed in early 2011 using a F5 robotic arm (Thermo Fisher Scientific, MA), on a four meter linear track specially designed for automated imaging and HCS; and was equipped with INCA2000, and INCA3000 for automated imaging, three MultiDrop Combis and three Elx405 plate washers for automated media swap for fixing or staining. The platform also included one Cytomat CO₂ incubator with a 504 plate capacity, one room temperature Cytomat hotel with a 189 plate capacity, one ABgene 3000 plate sealer and one LidPark lidding station.

E) Custom-built proprietary software

We had realized early on that the lack of a comprehensive informatics management structure would be a major bottleneck in our drug discovery efforts. At the time, building an informatics system in academic environment was very challenging, and no single out of the box commercial software solution was available to handle the diverse and growing needs of HTSCF. For this purpose, we opted back in 2003 to design, built, and implement a suite of modular applications, collectively referred to as the Oncology Research Informatics System (ORIS) to manage and support our data processing and storage requirements. ORIS is stable and efficient, used and verified by internal as well as external investigators. It is open-ended readily tunable system developed using open-source software, Java Enterprise Edition (J2EE) and JBoss with Oracle as a backend database because of its excellent reliability.

ORIS was developed as two distinct web applications, one for chemical screening (*Kemia*), and the second for RNAi Screening (*Genetica*).

In 2009 we developed our third GUI application that was a laboratory information management system called *Manus*, dedicated exclusively to handle day-to-day lab functionalities for example project chargebacks. *Manus* is also interactive with *Kemia* and *Genetica* backend databases to ensure data consistency and eliminate data redundancy across all our systems. Our current efforts are directed towards building an HCS image repository system, *Pyramus*, dedicated to handle HCS data. *Pyramus* is a collaborative effort with the teams at the MSKCC Data Center to develop a custom tailored image repository capable of secure image storage, handling and quick retrieval using a user-friendly web interface, with an ultimate goal of providing our investigators with an independent and secure access to the high content data outputs from their specific projects. Collectively, *Kemia*, *Genetica*, *Manus*, and *Pyramus* constitute the key constituents of our larger objective towards development of a comprehensive Oncology Discovery Explorer (ODE) system (Fig 6A).

HTSCF typical workflow and guidelines for projects

HTSCF is a full service facility providing a broad range of cost-effective services from assay development, primary screening, all the way up to confirmation of identified hits, requiring minimal contribution from the investigators (Fig 1B). We are open to work with customers all over the world in academia and research institutes as well as in Pharma or Biotech companies.

A) Project inception, evaluation, and planning

All research projects are considered on a case-by-case basis, following preliminary evaluation for suitability and amenability to screening. Access to the assay development and screening services provided by the HTSCF typically starts with an initial consultation with the facility Head. This allows the facility director to discuss with the investigator their proposed project and ascertain both technical complexities and amenabilities to the industrial set up of HTS. At this stage of primary consultation, the research goals, expectations with regards to outcome, experimental feasibility, merits, and associated risks are reviewed. This review establishes whether the project is still just an idea or preliminary conditions have been determined based on experimental data generated by the investigator's group. The HTSCF director summarizes the overall concepts of the proposed and incoming projects and presents the resulting list of HTS assays to the Oversight Committee. The committee reviews the projects and addresses any concerns for both technical complexities for adaptation to HTS and scientific merits of each project, resulting in a prioritized list of projects, at which point a project gets initiated at the HTSCF. All of the assays coming to the facility start in the "exploratory assay phase" and from there, progress to "feasibility assay phase" while undergoing substantial improvements and optimization, to finally reach the "screening assay phase" where the assay is fully industrialized and ready for screening.

After the preliminary step of project assessment and prioritization, a detailed project plan is carved out that includes cost evaluation, project milestones and potential dependencies. The

HTSCF utilizes the Microsoft (MS) Office Project Enterprise Server to create, record, manage, and co-ordinate multiple projects running at any given time in the facility. Each incoming project is assigned to a unique scientist as the project manager, who becomes the point of contact for the investigator. The scientist works in close collaboration with the facility staff members from the compound management and data management team who contribute their expertise to each project. Facility staff members work closely with each investigator through all stages of assay development, optimization, automation, and screening. Investigators are encouraged to make necessary arrangements with staff members to perform their experimental work. We generally require a chargeback arrangement for consultations, consumables, and equipment usage.

HTSCF has established ground rules on how each project is managed throughout its course. All project related communications between the investigator and the assigned scientist are documented and transmitted via email and all experimental steps are recorded in the lab notebooks on a daily basis. The investigator is required to be updated biweekly in connection to their project status, timelines and future steps, and one-on-one meetings are organized at the completion of each milestone in the project workflow so as to evaluate the data with the investigator, assess the expected outcomes, and to make a go/no-decision. All documents pertaining to a project are saved in a specified format on the shared drive, with a secure and automated backup. All reagents and materials for each project are maintained separately and detailed chargeback records are maintained based on the usage per reagent, consumables, instrument usage, data storage, personnel time, and recovery cost.

B) High-throughput and high-content screening

Each project undertaken at the HTSCF is submitted to a standard workflow meant to systematically optimize each parameter of the assay and validate the assay for screening. Following this workflow is necessary to ensure good performance of the assay in the high density format (384- or 1536-well microtiter plates) required for automation and industrialization of the assay, and for generation of high quality data during screening. The standard phases in a typical screening workflow are cell growth monitoring (for cell-based assays), assay transfer and miniaturization, control run, assay validation, primary screening and confirmation. Collectively, members of the HTSCF have extensive experience and individual expertise in a wide array of assay technologies covering cellular, biochemical, and affinity-based assays (Table 4).

At the time of project initiation for all cell-based assays, cells at a low passage number are requested from the investigator and after material transfer cells are cultured according to a standard tissue culture protocol in an antibiotic-free media in quarantine incubators for growth assessment, and cell stocks are also prepared (typically 25 frozen vials per cell line). Cells are typically grown in low-density environment continuously for 2 weeks and samples are tested for mycoplasma, bacterial and fungal contamination; and if deemed necessary cell authentication. Consistent contamination-free cell growth is the criteria of success at this stage at which point we proceed to the assay development phase. Our workflow provides investigators with confidence from the onset of project, screening, and confirmation of hits.

Chemical screening workflow—Chemical screening utilized the power of small molecules to help further elucidate known or discover novel cancer pathways through a systematic workflow (Fig 4A). Chemical screens, conducted at HTSCF, primarily fall under two categories: 1) cell-based assays (Fig 4B), and 2) target-based assays (Fig 4C). A typical chemical screening process is sub-divided into five broad steps, and takes on an average six months for its successful completion. The steps pertaining to cell growth and quality assessment are not applicable to target-based screens; besides, subsequent steps for screening are similar for both target-based and cell-based screens.

Assay development & optimization for automated screening: Great care is taken during assay development to identify conditions relevant to physiology and conditions leading to good assay sensitivity, allowing us to identify hits/actives during screening campaigns. For example, we investigate the following parameters for enzymatic target-based assays: substrate concentration with respect to the Michaelis constant (K_m), active enzyme concentration, incubation time, degree of substrate depletion, order of reagent addition and time dependence of compound activity. For binding assays we investigate: ligand concentration with respect to equilibrium dissociation constant (K_d), receptor concentration (for both soluble and membrane associated receptors) and order of reagent addition. For cell-based assays, observed response should be linear with respect to cell number in the well, pre-incubation of cells with test compounds is considered when applicable (e.g. assays in which an agonist or antagonist is added), and optimal incubation time should be selected in accordance to the rule of avoiding underestimation of inhibition or activation. The HTSCF has industrial scale and state of the art instrumentation in place and has the flexibility to accommodate most of the detection technologies used in the industry, performed in 384-well or 1536-well microtiter plates. While MSKCC investigators have sometimes already worked out conditions for their assays, the expertise provided by the HTSCF staff such as assay miniaturization and automation are crucial components of HTS, together with statistical analysis of assay quality.

During the process of assay transfer and miniaturization, HTSCF scientists optimize the assay conditions for translation to an assay amenable to high density format. For a high content assay, the first step consists in assessing the growth kinetics of the cells used in the assay in 384-well microtiter plates, leading to the selection of the optimal cell seeding density and incubation time. The dose response of known modulators is assessed and optimization of reagent concentration and time point for the assay may be performed at this stage, in combination with the identification of the best imaging platform and image analysis parameters. The observation of an expected response with the known modulators in the optimized assay is required before locking the assay conditions in the Standard Operating Procedure (SOP).

Control Run: Assay development is concluded by a control run performed on the automated platform in the conditions of screening and consisting of a significant number of high and low controls in triplicates to ensure that the statistical performance of the assay is compatible with high throughput screening, prior to moving forward with assay validation. For high-throughput and high-content assays that have passed our initial quality assessment

are first scaled up and tested against over 1,000 data points for high and low control to establish Z' factor, Signal-to-Noise Ratio (S/N), and coefficient of variance (CV). A Z' factor of >0.5 is representative of a good assay window. Data from the control run are also checked for systematic errors by heat map analysis. Assays that meet the HTSCF minimal requirements are then progressed to screening phase against our library collections.

Validation (Pilot screen): For small molecule screening, the assays are tested against our validation library comprised of approximately 7,000 compound collections of FDA approved, off-patent, and known bioactives. The validation library is screened in duplicate to assess range, robustness, and reproducibility. It is also a very good measure for the initial hit rate.

Primary screening: Primary screening is performed on automated platforms using the previously optimized conditions using the SKI corporate library of 400,000 compounds and at a screening concentration of 10 μ M, performed in duplicate. The assay readouts associated with the primary screen can be as simple as measuring alamar blue conversion in LEADseeker to as complex as mutliparametric with up to 10 features selected using the more sophisticated INCA microscopes. Plate columns 13 and 14 are generally reserved for control addition, and for simple assays measuring cellular viability, 1% DMSO (v/v) and Killer Mix (1 μ M) are the common choices for negative and positive control. For more specific assays, the selection of controls is performed by literature search, historic data and recommendations with the investigator. The analysis of high and low controls present on each plate ensures that the statistical performance of the assay was acceptable throughout the screen. Hit selection is based on activity of compounds across the screen. Initial hit rate, overlap analysis, chemical scaffold analysis and unbiased hit selection are performed by the data management team and consulted with MSKCC investigator. The hits obtained from the primary screen are further characterized based on their structure in a scaffold analysis.

Confirmation Studies: As a rule of thumb, the hits obtained from primary screen are first re-supplied from the vendor for follow-up experiments. We never cherry pick the hits from our chemical libraries as we do not want to comprise the integrity of our supply stocks. Confirmations studies include and are not limited to re-testing the selected hits, dose response, quench test, solubility test, interference tests, and cytotoxicity assessment. Confirmations are performed according to the pre-established SOP, that is using the same assay conditions, plate formats and detection reader, and the assay robustness is assessed using Z' factor.

Dose Response studies: The dose-effect relationship of the selected hits is evaluated using a dose response curve (DRC). A dose response is generally conducted using 12 doubling dilutions from 1 μ M, 10 μ M, and 100 μ M concentration ranges using the same assay workflow. These studies are performed in duplicate and in presence of controls to assess the reproducibility and robustness of the assay respectively. This data is loaded into ORIS and percentage inhibition values are calculated based on high and low controls on each plate. DRCs are fitted manually and IC₅₀ values for each compound are obtained. Finally, an

executive summary is compiled for the investigator, which also includes hits from the primary screen as well as the ones confirmed in the follow-up studies.

Hit to lead discovery: Once the potential hits have been identified through HTS, the next step is to progress these hits towards generation and optimization of leads for clinical drug development. Following successful completion of hit confirmation phase, the identified hits are re-synthesized and subjected to exploratory chemistry studies, where generally about 20 compounds per scaffold are assessed. The key considerations at this stage are the drug-likeness of the compound, target selectivity, tractability of hits, and intellectual property (IP) protection. The next step entails synthesis of new analogs for selected compounds, and requires expertise of medicinal chemists to ensure synthesis of compounds with high-quality and improved affinities; this step is a risky and expensive endeavor. An important consideration here for cost-effective solutions is to evaluate the cost of synthesis as full time equivalents (FTE) versus fee-for-service (FFS) on a compound-to-compound basis. Typically, we outsource our medicinal chemistry requirements to specialized companies in China, United Kingdom, and India. MSKCC claims complete ownership of all IP generated (Fig 4D).

RNAi screening workflow: RNAi screens are designed and implemented to achieve simultaneous gene-by-gene knockdowns, providing a great opportunity for quick and versatile functional genomic studies (Fig 5A). The two widely used RNAi technologies offered at HTSCF are synthetic siRNA duplexes in singles and plasmid based shRNA hairpins in arrayed formats; the choice of technology is by far context dependent. For example, hard to transfect cells are more amenable for HTS using the shRNA hairpin technology. Furthermore, the choice of technology is also driven by duration of perturbation required to observe a desired phenotypic effect, for transient silencing siRNA duplexes are given preference while for a more consistent and stable knockdown shRNA hairpins are preferred.

Our RNAi libraries are diverse in coverage giving us the capability to conduct screens in focused gene sets and up to a genome-wide scale. The RNAi screens conducted predominantly fall under three main categories: 1) Loss-of-function, 2) Gain-of-function, or 3) Drug modifier screens.

i) siRNA duplex screening workflow

Assay development & optimization for automated screening: For siRNA duplex screening projects, assay development is comprised of two steps performed in parallel which include documenting growth kinetics to determine optimal cell seeding density per well, and assessing optimal transfection efficiency (Fig 5B). All experimental steps are carried using automated instrumentation. Unless specified all assay are carried out using reverse transfection procedure containing 5uL/well of siRNA, 25uL/well of transfection reagent complex, and 50uL/well of cell suspension. Alamar Blue conversion (AB) and Hoechst nuclei staining (NUCL) are standard measures of cellular viability, quantified using LEADseeker and INCA2000 (4× magnification) respectively. Cell growth kinetics is monitored over the duration of the assay, typically 7 days, to document doubling

characteristics and consistent cell viability. Simultaneously, eight different transfection reagents are first tested for toxicity, out of which the top four transfection reagents are further assessed for forwards and reverse transfection efficiencies using Silencer® Select Negative Control (SNC), PLK1, and KIF11 siRNA duplexes with NucView, AB, and NUCL as readouts of choice; 5 time points starting from 24 hr post transfection are assessed. Best transfection conditions are determined based on: 1) the conditions leading to >8-fold difference between SNC and PLK1 or KIF11, and 2) <1.2-fold difference between mock transfection and SNC for cytotoxicity. Finally, the best transfection conditions are selected based on low cytotoxicity and highest transfection efficiency and the optimal transfection parameters are locked into an SOP.

For the drug modifier screens, an additional step of dose response is conducted using the compound of interest typically at two concentration ranges that of 1µM and 10µM over a time course experiment. At this step, we expect to observe a drug modifier behavior consistent with previously reported data and the drug concentration is selected based on assay specifics. Once the assay conditions are optimized, the SOP is finalized (Fig 5B).

Primary Screening: After completion of all preliminary steps of assay development and optimization, the final SOP is locked. Post approval, the primary screen is executed strictly adhering to the conditions locked in SOP. After the successful completion of the screens, the plates are imaged to obtain raw data and hit nomination is carried out using the Bhinder-Djaballah Analysis (BDA) method, which is an unbiased systematic workflow developed in-house specifically to analyze data outputs from RNAi screens [2]. Similar to chemical screens, columns 13 and 14 of a 384-well plate are reserved for control addition. SNC and PLK1 are routinely used negative and positive controls, respectively, for lethality screens. Multiplex assays generally comprise of assessing fluorescence via an enhanced green fluorescent protein (EGFP) reporter, NUCL to measure cell death and AB to measure cellular viability. For a genome-wide screen, there are up to 64,755 data points obtained from the primary screen.

ii) shRNA hairpin screening workflow

Assay development & optimization for automated screening: There are four primary considerations during assay development stages of an shRNA hairpin screen: 1) determine optimal cell density per well, 2) assess maximal polybrene concentration to be used for transduction, 3) assess minimal puromycin concentration to be used for enrichment of transduced cells at IC₅₀ and IC₉₅, and 4) identify optimal multiplicity of infection (MOI) for transduction by monitoring Turbo GFP signal expression (Fig 5C). As in siRNA duplex screen, AB and NUCL are the readouts of choice, and cell viability is assessed over the duration of the assay, which is typically 14 days. The four steps listed above are performed in parallel and the optimal conditions are locked in an SOP to be followed through during the course of validation and primary screening. For the drug modifier screens, dose response is conducted in a manner similar to that previously described for siRNA duplex screening. Seed cells using optimized cell seeding density in one positive control plate and one negative control plate, respectively, and assess cell viability by staining cells followed by imaging. Criteria for success are that the statistical performance of assay is acceptable as

using defined standards; assay is free of plate effects and systematic errors. Prior to industrialization of the assay, the assay is validated using known modulators or using generic modulators of cellular viability under the conditions established in the SOP. At this stage, the cells are scaled up and instruments are assessed for quality control in preparation of primary screening. Using our standard transduction cassette comprised of shRNA lentiviral control particles, transduction efficiencies are assessed based on performance of the controls and that of the known modulators of a cellular event relative to the controls measuring NUCL and fluorescence signal as readouts.

Primary Screening: After the necessary approvals, we execute the primary screen using the validated assay and pre-established conditions, closely following the SOP. After successful completion of the screens, the assay plates are imaged and quantified using the INCA microscopes. The raw data thus obtained is analyzed using the BDA method to nominated hit candidates that confer a measurable phenotypic perturbation in the screen (Fig 5C). As described earlier, plate columns 13 and 14 are reserved for control addition. The genome-wide TRC1 library has wells O₁₅₋₂₄-P₁₅₋₂₄ empty and serve as internal controls to assess baseline cell kill by puromycin alone. A genome-wide screen can yield up to 80,600 data points in the primary screen.

C) HTSCF data analysis and management practices

HTS data analysis and reporting—Data analysis and reporting are performed as a collaborative effort between the scientists assigned to the project and the data management team, with experience in the domains of cheminformatics and bioinformatics. Great care is taken during selection of an ideal hit selection methodology for each screen to ensure robust and reproducible outcomes. For the purpose of data analysis, we use custom built routines in R statistical programming language and Perl. We have also acquired and put to use several licensed enterprise software's, which include Sigma Plot (SYSTAT Software Inc., CA), SpotFire (TIBCO, CA), Marvin (ChemAxon, MA), and Metacore (Thomas Reuters, NY). In addition, we have an unlimited paid access to the Ingenuity Pathway analysis tool (Ingenuity Systems Inc., CA) made available through MSKCC.

As a part of HTSCF protocol, the investigators are apprised with the results and findings of their experiments at the completion of each milestone in the project. A control run and validation report is generated to summarize the results of assay optimization and validation in a pilot screen, followed by an executive summary report for the primary screen. A comprehensive screen and confirmation report is communicated to the MSKCC investigator at the conclusion of the project; at which point the raw and image data is also released for their evaluation.

Chemical screen data analysis—As a first step we assess the quality of the data generated using the quality control matrices. Ideally we would expect a Z' factor >0.50, S/N >3, and CV <20% as indicators of good screening data outputs. In addition the data is visually inspected with the aid of box and scatter plots, distribution plots, and heat maps to determine overall screen robustness. Individual plate maps are also reviewed to account for any spatial or systematic plate errors. Screens performed in replicates are assessed for their

reproducibility and good correlation; depending on which the replicates are averaged for the purpose of hit identification. The data outputs from chemical screens are generally transformed in to percent inhibition values relative to the high and low controls for further analysis and hit candidates are selected at a threshold generally determined at > 80% cell kill. For those selective screens conducted under the presence or absence of an external factor as an example +/- drug screens, hits are selected based on a simple linear regression model. Furthermore, for screens conducted in parallel against a panel of cell-lines, relative fold change values are calculated to characterize differential hit activity (Fig 4B-C).

RNAi screen data analysis—Initial steps to ensure data quality are performed in a manner similar to that described for chemical screening; at this stage the data variability and spread is assessed. Hit nomination for all RNAi screens is performed using a streamlined approach, the BDA method, pioneered at HTSCF, which involves a five-step workflow: 1) active duplex identification, 2) active gene identification based on an H score of 60, 3) OTE filtering, 4) re-scoring, and 5) biological classification (network building, functional annotations, and canonical pathways) [2]. Images of all hits are exported for quality control and visually inspected to filter out obvious false positives, resulting in a refined list of high-confidence hit candidates for follow-up (Fig5 B-C).

HTS and HCS data management—For the purpose of efficient screening data management, we load all data corresponding to any particular screen into our custom-built ORIS, comprised of *Kemia* for chemical screens and *Genetica* for RNAi screens. Broadly, ORIS is comprised of three main modules, ChemReg (Compound management), iPlate (plate management) and OpenHTS (screen data management) that are completely independent and replaceable (Fig 6A). The development and production servers for ORIS are physically located off-site at the MSKCC Data Center (Lyndhurst, NJ), which houses all our servers and databases, providing regular maintenance, and updates to ensure data integrity and security. The information systems unit located at MSKCC's SKI division, SKI Research Computing, extends great support in providing expandable data storage capacity for ease of storage and retrieval of HTS and HCS data; our current usage is about 105 TB, occupying about 17% of all the data on a shared MSKCC cluster (Fig 6B). In addition, *Manus* enables us to organize and manage the daily operations, including projects, targets, run requests, plate requests, orders, and detailed chargebacks (Fig 6A). Our well-integrated web-based applications allow multiple users to access the systems at the same time to register compounds, load data, query data, and maintain secure electronic records for smooth functioning.

For HCS, the assay plates are imaged on an INCA automated microscope and the image acquisition takes about 24 minutes per 384-well plate. The procured images are processed with the help of IN Cell Developer Toolbox using our custom-built image analysis protocols, including 2D imaging [3], enabling quantification of one or more cellular events at a rate of 4 seconds of processing time per image, producing mounds of images and associated metadata. As an example, for a typical genome-wide shRNA hairpin screen comprised of 295 assay plates, it takes about 6 days of continuous image acquisition time

yielding up to 113,280 images and approximately 1 TB of image information to manage, followed by up to 5.5 days of continuous image analysis.

HTSCF Capacity

The HTSCF has a mixed screening portfolio of chemical and RNAi, genome-wide as well as focused screens. Depending on the assay technology utilized, it would take from two weeks to forty weeks to conduct a complete HTS or HCS project. Within an annual quarter, the HTSCF has perfected its techniques and has become proficient on performing several screens simultaneously, using the various technologies and automated systems. Our capacity has increased substantially since the upgrade of the existing robotic platform (*Xanthus*) and the introduction of the second robotic platform (*Hestia*). This allows us to engage on 25 projects per year assuming that 5 projects would fail at the assay development stage, leaving us with a capacity to perform up to 20 screens per year.

HTSCF cost effectiveness

The HTSCF works in close collaboration with MSKCC scientists to provide them with services customized to their specific requirements, constantly exploring new assay technologies to accommodate their needs. In addition, by working on multiple projects simultaneously and coordinating screens, the Core conducts economies of scale as detailed below. The increased capacity allows the HTSCF to take on a larger number of projects per year, decreasing the wait time for prospective users and maximizing the use of our staff, equipment, space and automation for a better return on the investment from MSKCC. In turn, our high capacity positively impacts the cost of reagents and consumables. The HTSCF has built strategic relationships with various vendors by leveraging this high capacity to negotiate competitive pricing. The savings are passed on to the investigators that screen at the HTSCF. The large quantity of consumables ordered by our facility has even indirectly impacted the MSKCC community, by lowering the price by 10% to 30% for other laboratories ordering some of the same consumables as the HTSCF. In some cases, we have managed to maintain consistently low pricing on tissue culture consumables from past three years.

For screening reagents, ordering is done by directly negotiating with each vendor individually. We have been able to obtain 15% to 40% discount on media and reagents; in turn we pass those savings to the investigators screening at our facility. The expertise of the Core in assay miniaturization in 384- or 1536-well format is critical in enabling large screens that would be cost-prohibitive to run in standard 96-well format. As a rule of thumb, 384-well format enables savings in reagent up to 4-fold compared to 96-well, and up to 16-fold in 1536-well format. Miniaturizing the assay format also makes possible screens for which MSKCC investigators produce their own reagents such as recombinant proteins that they cannot produce in industrial amounts. In addition, the HTSCF has identified and works with several Chemistry CROs enabling chemistry synthesis for exploration of structure activity relationship analysis of obtained hits to be performed in a timely fashion and relatively low costs. Compared to screening facilities at other institutions, the HTSCF is

unique in that it provides a full service, with each step of the process performed by experienced HTSCF staff.

Chargeback mechanisms

The chargeback's offset a portion of the HTSCF's operating budget. Rates are set to provide an affordable fee for service that can be supported by the research funding of the user base. We charge for our services in four areas: 1) individual small molecule requests, 2) consumables, 3) use of instrumentation, and 4) HTSCF personnel recovery charges. Our custom built LIMS system helps to accurately create chargeback's and to maintain financial records. Although being a full service facility, our pricing remains at par with other some of the other HTS facilities, which serve as resource centers with an open lab use policy (Table 5).

Other Services & Expertise

A) Cell banking and cell line maintenance

For cell-based assays, we have implemented a standardized procedure to ensure the integrity of patient-derived, immortalized, and recombinant cell lines. Upon receipt of each cell line from our investigators, the cells are inspected for contaminants, documented, quarantined, and tested for mycoplasma infection. Cell lines are rapidly expanded to created stocks at the lowest available passage for assay development and the screening process. Currently, we have over 100 cell lines including panels for NSCLC, retinoblastoma, lymphoma, and rare tumor types such as liposarcoma. In terms of future expansion, we will be implementing a new state of the art automated biobanking for storage, retrieval, and testing of cell lines (Fig 7). To meet new standards in grant approvals and publication process, cell are authenticated by short tandem repeat (STR) analysis upon receipt and checked at regular intervals between passages to eliminate misidentified lines.

B) Drug combination studies

We have recently undertaken tremendous efforts to perform large-scale dose response studies to assess the overall synergistic effect of up to two drugs in combination. So far, we have successfully completed a drug combination project comprised of 18×5 drug combinations run in duplicate at $10\mu\text{M}$ and $2.5\mu\text{M}$ concentration ranges respectively, conducted in a 1536-well format for 12 cell lines in parallel using AB as readout. This endeavor resulted in 804 DRCs corresponding to the combinations under evaluation; IC_{50} 's for each curve were determined. Based on DRC's, the drug combinations that produced a significant shift in cell viability were select and further analyzed using the CompuSyn software (ComboSyn Inc., NJ), which calculates their combination index (CI). The CI served as a quantitative indicator enabling characterization of the observed effect as antagonistic, additive or synergistic. We believe that such an approach holds a great potential for targeted therapy to fight chronic diseases such as cancer.

C) Supply of small molecules

We accept requested pertaining to re-supply of individual small molecule chemicals for on-going projects and the compounds are re-supplied from the vendor at a discounted price. The

standard amount per compound is 10 mg quantity and any discount on price obtained by bulk ordering is passed on to the MSKCC investigators. A compound resupply charge is applied to cover the cost of handling.

D) Production of high-titer Lentiviral particles

For focused shRNA hairpin screening, we offer customized synthesis of high-titer HTS-ready lentiviral particle libraries. First, the investigators provide us with a list of genes from the TRC libraries, selected for a focused screen. In the next step, we order bacterial glycerol stocks from the vendor and culture the glycerol stocks in 384-well microtiter plates to a specific optical density for purification of plasmid DNA. We are equipped to generate high quality DNA plasmids enabling production of high-titer shRNA lentiviral particle libraries using high-throughput endotoxin-free DNA kits. The shRNA hairpin lentiviral particle libraries are arrayed in 384-well microtiter plate format and have viral titers of 1×10^7 particles/well.

HTSCF technology development initiatives

Guided by our core belief that efficacious cell-based drug discovery screening ought to be high throughput/high density amenable and high content with multiplexed readouts capable of single cell level analysis, we have successfully explored and established several technologies. Two of the highlights are receptor tyrosine kinase (RTK) biosensor assay and microRNA (miRNA) biosensor assay as described below.

RTK Biosensor Assay

Receptor tyrosine kinase (RTK) receptors are a category of important targets for cancer therapeutics. Several small molecule RTK inhibitors with encouraging outcomes in the clinic include Gefitinib and Erlotinib that target epidermal growth factor receptor (EGFR) for non-small cell lung cancer (NSCLC), Imatinib (Gleevec, Novartis) that targets BCR-Abl tyrosine kinase, platelet derived growth factor receptor (PDGFR), and c-KIT for chronic myelogenous leukemia and gastrointestinal stromal tumors, and Sorafenib (Nexavar, Bayer and Onyx) that targets Vascular Endothelial Growth Factor Receptors (VEGFRs), PDGFR- β , and FLT3 for renal-cell carcinoma. However, *de novo* escape mutations have rendered invariable resistance to RTK inhibitors, calling the need for the discovery of novel inhibitors. Instead of following the conventional approach of RTK inhibitor identification using recombinant purified kinase domains *in vitro* against a panel of kinase-focused chemical libraries, we have strategized a novel approach of using domain-based biosensor platform to assess the effect of input signal on endogenous EGFR activity taking into account of both EGFR activation and internalization, therefore providing a more comprehensive data set-based analysis. Through scientific collaboration with Sigma-Aldrich, we developed a 2 $\times$ -SRC homology 2 (SH2) domain-based EGFP biosensor system, in which SH2 from the adaptor molecule Grb2 binds to tyrosine phosphorylated EGFR with high affinity upon epidermal growth factor (EGF)-triggered EGFR dimerization and activation [4]. Specifically, upon ligand binding, EGFR activation and phosphorylation recruits fluorescent SH2-domain based biosensor followed by receptor endocytosis and recycling. Receptor clustering and localization can be visualized and quantified using high

content imaging methodologies in live cells. A significant advantage of our platform over existing ones is that it allows endogenous EGFR function to be investigated in its natural environment to most closely mimic the *in vivo* reality. More importantly, our successful application of the EGFR domain-based biosensor platform in compound library screen has led to the discovery of previously unappreciated hits as crucial regulators of ligand-induced EGFR activity and function in addition to known compounds [5]. We have also explored the application in RNAi library screens and have identified promising molecular determinants modulating EGFR activity. We are currently working on extending domain-based RTK biosensor to additional key RTKs as well as key nodal points of downstream RTK signaling pathways and networks.

microRNA (miRNA) Biosensor Assay

MiRNAs are small, single-stranded, and non-coding RNAs that have now been recognized as playing an important role in post-transcriptional regulation of gene expression via complementary base pairing to targeting messenger RNA (mRNA) at the 3'-UTR. miRNAs are versatile gene expression regulators that an individual miRNA can control the expression of up to hundreds of mRNAs. It is estimated that approximately 30% of the genome is under the regulation of miRNA during development and in diseases especially in cancer whereby miRNAs play a dual role of oncogenes and tumor suppressor genes. These unique properties of miRNAs render them attractive therapeutic targets. To better understand miRNA action in gene expression regulation, it is of paramount importance to study miRNA biogenesis. Over the years concerted efforts have been devoted to this field of study, yet largely based on hypothesis-driven approaches to study one miRNA at a time. In light of this methodology deficiency, we have developed a systemic high throughput platform to profile modulators of miRNA-21 in genome-wide campaigns. We first established a miRNA21-EGFP stable biosensor cell line by engineering a construct in which EGFP expression is under the control of miRNA via 3'-UTR complementarity. In response to input signal, EGFP expression either becomes liberated upon miRNA21 repression or remains in check when the input signal has no effect on miRNA21 expression. To test the effectiveness of our biosensor system, we applied it to four large-scale screens including a compound screen of 6,912 bioactives, a miRNA mimic screen, a siRNA screen targeting 21,565 genes of the human genome, and a shRNA screen covering 16,039 genes of the human genome [6-8]. Using high content image acquisition and analysis methods highlighted by single cell level analysis, we have identified, in addition to known genes that regulate miRNA biogenesis, novel compounds and genes that significantly boosted EGFP signal intensity gain as a result of miRNA-21 repression. Systemic pathway and network of these hits collectively has served to enrich our current understanding of mechanistic details governing miRNA biogenesis. Our proof-of-principle biosensor approach has exciting applicability to investigating biogenesis and their biological consequences of additional key miRNAs.

HTSCF Research support & collaboration with MSK clinicians: Towards a common goal of translational medicine

HTSCF has always extended support towards translational research helping investigators seeking grants for funding their research projects and collaborated on many projects

resulting in numerous publications. Some of the examples of our successful collaborations and contributions towards peer reviewed funded projects are detailed below.

Drug repurposing to fight disease

The new approach of intra-arterial chemotherapy opened doors to explore the chemotherapeutic agents previously neglected due to their high systemic toxicity. In collaboration with Dr. David Abramson (Chief, Ophthalmic Oncology Service, MSKCC), we took this as an opportunity to revisit the FDA-approved drugs and known bioactives that could be administered by local intra-arterial infusion for the treatment of retinoblastoma. To this end, we developed and successfully implemented a chemical screen using 2,640 FDA-approved drugs and bioactive compounds against two retinoblastoma cell lines Y79 and RB355 [9]. The hits identified in the screen shared a common chemical scaffold resembling the core structure of previously characterized cardenolides. The potency of the selected hits was confirmed in a panel of ocular cancer cell lines. Furthermore, we investigated the therapeutic effects of the cardenolide ouabain at the Antitumor Assessment Core Facility (MSKCC) using a xenograft model of retinoblastoma and complete tumor regression in the treated mice was observed [9]. At Dr. David H. Abramson's clinic, cardenolide digoxin, identified as one of the hits from HTS, was directly translated into clinical use. After seeking approval under an Investigational New Drug for compassionate use, digoxin was administered to a 4-year old boy with stage Vb retinoblastoma. Intra-arterial therapy produced a modest but measurable regression in tumor size but subsequent oral administration of digoxin produced no effect, most likely due to the current challenge in obtaining a sustained intraocular concentration of the drug [10].

First HTS amenable assay in human Embryonic Stem Cells (hESCs)

Development of assays with feasibility for adaption to HTS have been particularly challenging for hESCs due to difficulties in establishing ideal growth conditions. We, for the first time, reported on a validated assay for hESCs and demonstrated its successful industrialization to HTS using 2,880 compounds in multiple replicates, to identify compounds that promote self-renewal and differentiation [11].

Mutant EGFR-Dependent Lung Cancer in Human Cell Lines

The aim was to develop cell-based cytotoxicity assays against a panel of six NSCLC cell lines and to perform screening using four cell lines with varying degrees of sensitivity to the EGFR inhibitor Tarceva; in order to identify small molecule inhibitors of cellular growth overcoming KRAS mutations. We also developed secondary cell-based cytotoxicity assays against a panel of 30 NSCLC cell lines harboring KRAS mutations and use them to screen the obtained hits from the primary HTS. The screening efforts led to the identification and characterization of four classes of small molecules overcoming KRAS mutations [12, 13]. Efforts are ongoing to identify the molecular targets for each of the four classes [12].

Small molecule screen to identify inhibitors of cytochrome c-mediated caspase activation

To miniaturize and adapt to HTS a biochemical assay recapitulating the cytochrome c-mediated caspase activation pathway, we screened a collection of 300,000 plus compounds

to identify inhibitors of caspase activation as chemical probes to study caspases and as potential therapeutic agents for neurodegeneration, stroke, radiation syndrome, and immune disorders. Follow up and co-crystallization studies suggest that a class of compounds identified in the screen constitute non peptidic allosteric caspase inhibitors that inhibit apoptotic and inflammatory pathways in cells [14].

Whole well imaging to screen for reversers of oncogenic transformed phenotype

To screen for compounds, which would reverse the transformed phenotype in live cell high content assays, a robust high content assay and a robust image analysis method need to be developed and validated in a model system. We have developed a whole well high content imaging assay to screen for reversers of the transformed phenotype in NIH-3T3 cells using the recently discovered oncogene KP and HRAS as model systems. This is the first example of an assay to study signaling in transformed phenotype. We developed a sensitive and robust assay [3]; we have devised image analysis algorithms sensitive enough to distinguish between monolayer of cells versus clusters or cell pileups. We completed a screen against a 6,000 compound library and a 56-member focused library identifying known inhibitors of PDGFR- β function as well as some novel modulators of transformed phenotype. We have also performed similar screen using HRAS as an oncogene in NIH-3T3 cells and found several differences in signaling pathways and their inhibitory effects on reversing the transformed phenotype. Identified hits are being mechanistically studied in the investigator's lab.

Small molecule screen to identify inducers of differentiation in liposarcoma

The aim of this project was to screen for small molecules that inhibit cell proliferation, induce apoptosis or drive differentiation of soft-tissue sarcoma; to develop a high content multi-parametric assay to identify such compounds that quantifies nuclei count as a measure of cell proliferation, caspase-3 activation as induction of apoptosis, and CEBP α adipogenic markers for differentiation. Using this multiplexed assay we successfully screened a collection of 3,000 bioactive compounds against three liposarcoma lines (DDLs, LPS, WD) and pre-adipocytes as control cell line for toxicity. In addition, we screened a larger collection of 21,000 natural products using time course measurements. Several promising compounds identified in the first screen [15] as wells as several natural products are currently undergoing follow-up characterization in the Singer lab.

Small molecule screen for inhibitors of the methyltransferases

We have developed and adapted a scintillation proximity imaging based assay (SPIA) for a panel of protein methyltransferases (PMTs) involved in post-translation methylation of various protein substrates and dysregulated in a variety of cancer types, in order to identify a new class of antiproliferative agents. This original assay was miniaturized and industrialized in a homogenous SPIA format amenable to HTS and screened against a panel of PMTs a 7,000 compound library representing a diverse class of small molecules that includes FDA approved drugs. We validated our SPIA screening strategy for PMTs by successfully screening four PMTs (SET7/9, SET8, SETD2, EuHMTase1) and identified specific PMT inhibitors [16] currently undergoing follow-up studies in the Luo lab.

Future vision: technological developments

As recently as the mid-1990's, most cell-based assays were not amenable to HTS. However, with recent technological advances in cell handling and detection, cell-based assays now make up a reasonable proportion of screens performed across the industry today. Imaging systems, on the other hand, have been developed to quantify cellular and sub-cellular fluorescence in whole cells. These systems have the capability of bringing detailed assays with high content information to screening, and enabling a better understanding of the effects of small molecules as well as gene knockdown by RNAi technologies. Automated imaging allows evaluating and quantifying phenotypes using high content analysis. Multiplexed assays can be performed at the same time in the same well and interrogating different markers results to record drug or gene knockdown signatures more accurately. In addition, high-content assays afford the added bonus of overcoming the noise induced by heterogeneous cell populations by providing access to cell-by-cell analysis. This is of tremendous importance in light of the more and more recognized importance of cancer heterogeneity. Instead of measuring the average response of a heterogeneous population, cell-by-cell analysis affords much improved accuracy. In addition, as advances in cell engineering allow combining multiple cell types to closely recapitulate a tissue function, the availability of complex cellular models will dramatically improve the significance of data generated *in vitro*.

Following this trend, several investigators at MSKCC have a strong interest in developing assays relying on the co-culture of different cell types, and/or in complex multi-cellular formations such as spheroids. We have already worked with several investigators to develop such assays and as an example we have pioneered methodologies allowing screening for small molecules able to revert cell transformation leading to the formation of cell clusters. Key to the success of our HCS efforts is the availability of the INCA2000 epifluorescence microscope allowing whole well automated epifluorescence imaging, and of the INCA3000 laser scanning confocal microscope; and most importantly their recent integration to the *Hestia* linear track robotic platform that enables fully automated screening.

We have observed over the past few years an increased demand for HCS due to its many advantages, as highlighted above. We anticipate a continuation of this trend, and especially a broad appeal for live cell imaging, enabling to record multiple time points for the same well. This approach enables to record a whole new dimension during screening: the evolution of cell response over time. In addition to providing the investigator with a richer and more accurate data set, live imaging saves on costs, as only one well is needed for multiple time points as opposed to performing multiple screens for multiple time points. This provides tremendous cost savings related to the purchase of microtiter plates, reagents, as well as instrument use. For this reason, we have considered expanding our current capabilities by acquiring an INCA6000, which would provide huge improvements in image quality and acquisition time, especially important for live cell imaging. As mentioned earlier, we foresee a growing trend towards HCS, and as an effort to preempt a potential rise in high throughput requirements, we plan to acquire a second INCA2000 to be integrated to the *Hestia* platform. Briefly, we take great care to review and undertake strategic measures to ensure that our facility maintains its high standards in infrastructure and expertise, and to provide

quality services to all our investigators. We are strongly motivated to drive innovation towards our ultimate mission of drug discovery and take necessary steps within reach to accelerate such efforts in the academic sector.

Acknowledgments

The HTSCF is partially supported by Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research, the Experimental Therapeutics Center of MSKCC, the William Randolph Hearst Fund in Experimental Therapeutics, the Lillian S Wells Foundation and by an NIH/NCI Cancer Center Support Grant 5 P30 CA008748-44. We also thank Wenjing Wu, Medical Graphics, MSKCC for her help with the artwork.

ABBREVIATIONS

AB	Alamar Blue
APL	assay plates
BDA	Bhinder-Djaballah Analysis method
CCD	charge-coupled device
DMSO	dimethyl sulfoxide
DPL	daughter plates
DRC	Dose Response Curve
EC50	half maximal effective concentration
EGFP	Enhanced Green Fluorescent Protein
esiRNA	endoribonuclease-prepared siRNA
H score	hit rate per gene score
HCS	High Content Screening
HTS	High-Throughput Screening
HTSCF	High-Throughput Core Screening Facility
IC₅₀	half maximal inhibitory concentration
INCA	IN Cell Analyzer
IP	Intellectual property
IPL	intermediate plates
K_d	equilibrium dissociation constant
K_m	Michaelis constant
miRNA	microRNA
MOI	Multiplicity of Infection
MSKCC	Memorial Sloan-Kettering Cancer Center
NSCLC	non-small cell lung cancer
NUCL	Nuclei Count

OTE	off-target effect
PDGFR	Platelet Derived Growth Factor Receptor
PMT	photomultiplier tube
RNAi	RNA interference
RTK	receptor tyrosine kinase
S/N	Signal-to-Noise Ratio
siRNA	small interfering RNA
SKI	Sloan-Kettering Institute
shRNA	short hairpin RNA
SOP	Standard Operating Procedure
SNC	Silencer ® Select Negative Control
SPL	source plates
TRC	The RNAi Consortium
VEGFR	Vascular Endothelial Growth Factor Receptors

REFERENCES

1. Radu C, Adrar HS, Alamir A, Hatherley I, Trinh T, Djaballah H. Designs and concept reliance of a fully automated high-content screening platform. *J Lab Autom.* 2012; 17(5):359–369. [PubMed: 22797489]
2. Bhinder B, Djaballah H. A simple method for analyzing actives in random RNAi screens: introducing the “H Score” for hit nomination & gene prioritization. *Comb Chem High Throughput Screen.* 2012; 15(9):686–704. [PubMed: 22934950]
3. Ramirez CN, Ozawa T, Takagi T, Antczak C, Shum D, Graves R, Holland EC, Djaballah H. Validation of a high-content screening assay using whole-well imaging of transformed phenotypes. *Assay Drug Dev Technol.* 2011; 9(3):247–261. [PubMed: 21182456]
4. Antczak C, Bermingham A, Calder P, Malkov D, Song K, Fetter J, Djaballah H. Domain-based biosensor assay to screen for epidermal growth factor receptor modulators in live cells. *Assay Drug Dev Technol.* 2012; 10(1):24–36. [PubMed: 22280060]
5. Antczak C, Mahida JP, Bhinder B, Calder PA, Djaballah H. A high-content biosensor-based screen identifies cell-permeable activators and inhibitors of EGFR function: implications in drug discovery. *J Biomol Screen.* 2012; 17(7):885–899. [PubMed: 22573732]
6. Shum D, Bhinder B, Djaballah H. Modulators of the MicroRNA Biogenesis Pathway Via Arrayed Lentiviral Enabled RNAi Screening for Drug and Biomarker Discovery. *Comb Chem High Throughput Screen.* 2013 Epub ahead of print.
7. Shum D, Bhinder B, Ramirez CN, Radu C, Calder PA, Beauchamp L, Farazi T, Landthaler M, Tuschli T, Magdaleno S, Djaballah H. An arrayed RNA interference genome-wide screen identifies candidate genes involved in the MicroRNA 21 biogenesis pathway. *Assay Drug Dev Technol.* 2013; 11(3):191–205. [PubMed: 23153064]
8. Shum D, Bhinder B, Radu C, Calder P, Ramirez CN, Djaballah H. An image-based biosensor assay strategy to screen for modulators of the microRNA 21 biogenesis pathway. *Comb Chem High Throughput Screen.* 2012; 15(7):529–541. [PubMed: 22540737]
9. Antczak C, Kloepping C, Radu C, Genski T, Müller-Kuhrt L, Siems K, de Stanchina E, Abramson DH, Djaballah H. Revisiting old drugs as novel agents for retinoblastoma: in vitro and in vivo

- antitumor activity of cardenolides. *Investigative Ophthalmology and Visual Science*. 2009; 50(7): 3065–3073. [PubMed: 19151399]
10. Patel M, Paulus YM, Gobin YP, Djaballah H, Marr B, Dunkel IJ, Brodie S, Antczak C, Folberg R, Abramson DH. Intra-arterial and Oral Digoxin Therapy for Retinoblastoma. *Ophthalmic Genetics*. 2011; 32(3):147–150. [PubMed: 21446853]
 11. Desbordes SC, Placantonakis DG, Ciro A, Socci ND, Lee G, Djaballah H, Studer L. High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. *Cell Stem Cell*. 2008; 2(6):602–612. [PubMed: 18522853]
 12. Somwar R, Shum D, Djaballah H, Varmus H. Identification and preliminary characterization of novel small molecules that inhibit growth of human lung adenocarcinoma cells. *J Biomol Screen*. 2009; 14(10):1176–1184. [PubMed: 19887599]
 13. Somwar R, Erdjument-Bromage H, Larsson E, Shum D, Lockwood WW, Yang G, Sander C, Ouerfelli O, Tempst PJ, Djaballah H, Varmus HE. Superoxide dismutase 1 (SOD1) is a target for a small molecule identified in a screen for inhibitors of the growth of lung adenocarcinoma cell lines. *Proc Natl Acad Sci USA*. 2011; 108(39):16375–16380. [PubMed: 21930909]
 14. Feldman T, Kabaleeswaran V, Jang SB, Antczak C, Djaballah H, Wu H, Jiang X. A Class of Allosteric Caspase Inhibitors Identified by High-Throughput Screening. *Mol Cell Biol*. 2012; 47(4):1–11.
 15. Angeles CV, Ugras S, Gobble R, Decarolis P, Shum D, O'Connor R, Djaballah H, Schwartz GK, Singer S. SN-38 is a potential targeted chemotherapeutic for dedifferentiated liposarcoma. Manuscript in preparation.
 16. Ibanez G, Shum D, Blum G, Bhinder B, Radu C, Antczak C, Luo M, Djaballah H. A high throughput scintillation proximity imaging assay for protein methyltransferases. *Comb Chem High Throughput Screen*. 2012; 15(5):359–371. [PubMed: 22256970]

A)

The Mortimer B. Zuckerman Research Center

B)

Figure 1.

Overview of the HTSCF at MSKCC and its contribution to the research community A) The HTSCF is located on the 19th floor of the Mortimer B. Zuckerman Research Center in New York. B) A standardized six-step workflow of a typical HTS screening project initiated at HTSCF. (mgmt, management)

Figure 2.
The HTSCF governance, organization and management schema

Figure 3.
Integrated robotics platform at HTSCF.

Xanthus is a Thermo Scientific F3 is an articulated robot on a 5m linear track, controlled by Polara v2.3 software. *Isis* is an Orbitor RS Microplate Mover, proving industrial-size performance yet compact to be integrated on any bench instrument. The Orbitor is a bi-directional telescoping arm with unlimited base rotations within a 360° workspace controlled by the Momentum v2.0.2. *Hestia* is an F5 articulated robot on a 4m linear track, controlled by Momentum v2.0.1.

A)

B)

Cell-based Chemical Screening Workflow

Assay development & optimization	<u>Phase I: Cell growth monitoring</u> <ul style="list-style-type: none"> Cells must be free of contaminants Cells exhibit consistent growth Frozen stocks prepared 	2 weeks
	<u>Phase II: Optimization</u> <ul style="list-style-type: none"> Doubling time and growth characteristics documented Initial cell seeding density obtained Drug titration for drug modifier assays performed Conditions locked in SOP 	1 month
Control Run	<ul style="list-style-type: none"> Statistical parameters (Z', S/N) assessed Dispensing instruments validated 	2 weeks
Assay Validation	<ul style="list-style-type: none"> Assay reproducibility & robustness tested Known agents & pan-active compounds identified Members of same scaffold identified consistently Initial hit rate compatible with screening 	1 month
Primary Screen	<ul style="list-style-type: none"> Primary screen in automated platform Raw data / Image acquisition Data QC & analysis (percent inhibition) Positive selection & overlap analysis Scaffold analysis & executive summary report prepared 	2 months
Confirmatory studies	<ul style="list-style-type: none"> Selected compounds are re-ordered from the vendors Perform confirmatory studies: <ul style="list-style-type: none"> <u>Confirmation</u>: Positives re-tested adhering to SOP <u>Dose Response</u>: DRC fitted & IC₅₀ at 1µM & 10µM <u>Quench test</u>: for Optical interference <u>False positive test</u>: for Compound interference <u>Solubility limit measurement</u>: for Compound solubility <u>Cytotoxicity assay</u>: Determination of EC₅₀ 	1 month

C)

Target-based Chemical Screening Workflow

Assay development & optimization	<i>Phase I: Optimization</i> <ul style="list-style-type: none"> Established protocol from collaborator obtained Translated volumes & combined steps for 384-well format Established linear range of detector Determined K_d, if applicable Optimal control identified Time course performed to improve S/N or signal stability Explore feasibility for 1536-well format Compare CCD and PMT based readouts Lock conditions in written SOP 	2 weeks
Control Run	<ul style="list-style-type: none"> Statistical parameters (Z', S/N) assessed Dispensing instruments validated 	2 weeks
Assay Validation	<ul style="list-style-type: none"> Assay reproducibility & robustness tested Known agents & pan-active compounds identified Members of same scaffold identified consistently Initial hit rate compatible with screening 	1 month
Primary Screen	<ul style="list-style-type: none"> Primary screen in automated platform Raw data / Image acquisition Data QC & analysis (percent inhibition) Positive selection & overlap analysis Scaffold analysis & executive summary report prepared 	2 months
Confirmatory studies	<ul style="list-style-type: none"> Selected compounds are re-ordered from the vendors Perform confirmatory studies: <ul style="list-style-type: none"> <u>Confirmation</u>: Positives re-tested adhering to SOP <u>Dose Response</u>: DRC fitted & IC_{50} at $1\mu M$ & $10\mu M$ <u>Quench test</u>: for Optical interference <u>False positive test</u>: for Compound interference <u>Solubility limit measurement</u>: for Compound solubility <u>Cytotoxicity assay</u>: Determination of EC_{50} 	1 month

D)

Figure 4.

Chemical screening workflow followed at the HTSCF

A) A broad overview chemical screening workflow, key features, identification of potential hits and their fate after discovery in HTS. **B)** A typical workflow for cell-based chemical screens. **C)** A typical workflow for target-based chemical screens. The standard HTSCF workflow for a small molecule chemical screen is divided in five primary stages; each stage has a list of deliverables and criteria to ascertain its success, and average time frame allocated for completion of each stage. **D)** A typical workflow to demonstrate the fate of hits identified in HTS and their progression into clinical leads.

(SOP, Standard Operating Procedure; CCD, Charge-Coupled Device; PMT, Photomultiplier Tube; K_d , Equilibrium Dissociation Constant; S/N, Signal-to-Noise Ratio; Z' , Z-prime Factor; QC, Quality Control, IC_{50} , half maximal inhibitory concentration; DRC, Dose Response Curve; EC_{50} , Half Maximal Effective Concentration; IP, Intellectual Property)

A)

B)

siRNA Duplex (Arrayed) Screening Workflow

Assay development optimization	<u>Phase I: Cell growth monitoring</u> <ul style="list-style-type: none"> • Cells free of contaminants • Consistent growth for 7 days • Frozen stocks prepared 	1 month
	<u>Phase II: Optimization</u> <ul style="list-style-type: none"> • Doubling time and growth characteristics documented • Initial cell seeding density obtained at 40-60% confluence • Drug titration for drug modifier assays performed • Toxicity of 8 transfection reagents assessed • Forward & reverse transfection efficiencies tested • Optimal transfection conditions locked in SOP • Dispensing instruments validated 	2 -3 months
Primary screen	<ul style="list-style-type: none"> • Primary screen on automated platform as per SOP • Raw data acquisition • Image acquisition 	1 month
Data analysis & reporting	<ul style="list-style-type: none"> • Data QC • Hit nomination (BDA method) • Images of hits retrieved for visual inspection • Comprehensive summary report prepared 	1.5 months

C)

shRNA Hairpin (Arrayed) Screening Workflow

Assay development optimization	<u>Phase I: Cell growth monitoring</u> <ul style="list-style-type: none"> • Cells free of contaminants • Consistent growth for 14 days • Frozen stocks prepared 	1 month
	<u>Phase II: Optimization</u> <ul style="list-style-type: none"> • Doubling time and growth characteristics documented • Initial cell seeding density obtained at 90% confluence • Drug titration for drug modifier assays performed • Polybrene kill curve assessed; max conc. selected • Puromycin conc. selected at IC₉₅ of the assay • Optimal MOI recorded • Optimal conditions locked in SOP • Dispensing instruments validated 	2 -3 months
Primary screen	<ul style="list-style-type: none"> • Primary screen on automated platform as per SOP • Raw data acquisition • Image acquisition 	1 month
Data analysis & reporting	<ul style="list-style-type: none"> • Data QC • Hit nomination (BDA method) • Images of hits retrieved for visual inspection • Comprehensive summary report prepared 	1.5 months

Figure 5.

RNAi screening workflow followed at the HTSCF

A) A broad overview of identifying promising targets in RNAi screening, inclusive of their follow-up and potential applications. **B)** A typical workflow for siRNA duplex screens. **C)** A typical workflow for shRNA hairpin screens. All steps and key considerations along with the average time frames associated with each stage of screening workflow are listed. (qRT-PCR, Quantitative Real-time Polymerase Chain Reaction, SOP, Standard Operating Procedure; QC, Quality Control, BDA; Bhinder-Djaballah Analysis; MOI, Multiplicity of Infection; IC₉₅, 95 percent inhibitory concentration)

A)

B) **Figure 6.**

Data analysis, management and storage capabilities at the HTSCF

A) ODE as an integrated platform with multiple data handling and storage capabilities; comprised of ORIS, which is adapted to handle chemical screening data (*Kemia*) and RNAi screening data (*Genetica*), and *Manus* is a lab information management system dedicated to handling administrative operations of the facility. **B)** Collaborations between the HTSCF, MSKCC Data Center and MSKCC's SKI Research Computing division enable efficient storage and backup of screening related data using secure connection. (mgmt, management; cpd, compound; str, structure; seq, sequence; IC₅₀, half maximal inhibitory concentration)

Figure 7.

A standardized workflow to establish and maintain a cell bank at the HTSCF

Table 1

List of diverse chemical library collections available at HTS ranging from FDA approved small molecules to natural products

Supplier	Library Collection
AnalytiCon Discovery	Natural products
Chembridge	Kinase, GPCR, Historic, Pharmacophore
Magellan BioScience	Deep sea extracts
NCI	Diverse small molecules; Natural Products
ChemDiv	Diverse small molecules
Specs	Kinase Inhibitors, Diverse small molecules
MicroSource	FDA-approved & known bioactives
Prestwick Chemicals	FDA-approved & known bioactives
SeqChem (Sequoia)	FDA-approved & known bioactives
Tocris Bioscience	FDA-approved & known bioactives
Signa-Aldrich	FDA-approved & known bioactives
Selleck Chemicals	FDA-approved & known bioactives
GSK	Kinase Inhibitors
Sarawak Biodiversity Centre	Plant extracts, Fungal extracts, Actinomycetes

NCI, National Cancer Institute, GSK, GlaxoSmithKline

Table 2

List of siRNA duplex and shRNA hairpin library collections available for HTS and HCA at the HTSCF

Type	Format	Vendor	Library Collection	Coverage	Organism	# genes	# duplexes
siRNA duplexes	Singles	Ambion® Life Technologies	Silencer® Select V4.0	Genome-wide	Human	21,565	64,755
			Custom built	Chromatin Modifying Enzymes	Human	127	381
			Custom built	DNA Damage Response genes	Human	257	771
			Silencer® Select V4.0	Kinases	Human	710	2,130
			Silencer® Select V4.0	Phosphatases	Human	299	894
			Custom built	Druggable	Human	6,363	20,742
shRNA hairpins	Arrayed	MSKCC	Custom built	Kinases	Human	779	2,244
			MISSION® siRNA Druggable Genome	Druggable	Human	6,598	19,755
			MISSION® TRC1	Genome-wide	Human	16,039	80,598
			MISSION® TRC1.5	Genome-wide	Mouse	15,518	77,128
			MISSION® LentiExpress™ Kinases	Kinases	Human	3,351	17,515
			Custom built	Epigenetic genes	Mouse	138	676
miRNA	Arrayed	Sigma-Aldrich	MISSION® microRNA Mimic	miRNA mimics	Human	654	3,102
			Custom built	Epigenetic genes	Human	461	2,407
esiRNA	Arrayed	Sigma-Aldrich	MISSION® esiRNA	Kinome	Human	N/A	884
shRNA, Short Hairpin RNAI;	Arrayed	Sigma-Aldrich	MISSION® esiRNA	Kinome	Human	515	518

shRNA, Short Hairpin RNAI; siRNA, Small Interfering RNA; miRNA, MicroRNA; esiRNA, Endoribonuclease-prepared siRNA

Table 3
List detection technologies and corresponding instrumentation available at the HTSCF

Detection	Readout	Instrumentation
Absorbance	Absorbance	SpectraMax® Plus384
Fluorescence	Fluorescence intensity	VICTOR3 V™ Multi label counters
	Fluorescence resonance energy transfer	
	Fluorescence intensity distribution	LEADseeker™ Multimodality Imaging System
	Fluorescence polarization	
Radioactivity	Time resolved fluorescence	Wallac Microbeta® TriLux counter
	Scintillation Counting	
	Filter Binding technology	
	FlashPlate technology	
Luminescence	ScintiPlate technology	LEADseeker™ Multimodality Imaging System
	Chemiluminescence	
Nephelometry	Laser nephelometry	Nephlostar
Imaging	Fluorescence expression, granularity, trafficking, translocation, nuclei, and cell count	INCA1000
		INCA2000
		INCA3000
Liquid Handlers	Compound and serial dilutions	MultiProbe II Plus
	Plate replication compatible with 96, 384, & 1536-well applications	Personal Pipettor
Robotics	Screen automation	Xanthus
		Hestia
		Isis
Informatics	Data Storage	Production & Test Servers

INCA, IN Cell Analyzer

Table 4

Characterization of diverse range of assay technologies available at the HTSCF

Category	Assay type	Assays technologies	Mechanism
Cell-based (Low content)	Cell viability	Alamar Blue, Calcein AM	Reduction of reagent by mitochondria, reagent cleavage by esterases
	Cell proliferation	CellTiter-Glo	Measure ATP by bioluminescence
Cell-based (High content)	Trafficking	Nuclear Cytoplasmic Translocation Assay	Internalization, intracellular re-localization
	Biosensor	Granularity Assay	Surface marker expression, kinetic marker expression, exocytosis
	Object intensity	Fluorescence Assay, Caspase Assay	Fluorescence readout
	Cell proliferation	Nuclei and Cell Count Assay, Calcein AM	Nuclear and cellular staining
Biochemical	Enzymatic	AlphaLisa Assay, Malachite Green Assay, ADP-Glo Assay	Kinases, proteases, deformylases; phosphatases, helicases, dehydrogenases, transferases, ATP-ADP production
	Binding	Fluorescence Polarization Assay, HTRF Assay	Soluble receptor:ligand interaction, membrane associated receptor:ligand interaction, RNA:protein interaction, DNA:protein interaction, protein:protein interaction
	Radioactive	Scintillation Proximity Assay	Methyltransferases, phosphorylation

ATP, Adenosine Triphosphate; ADP, Adenosine Diphosphate; Calcein AM, Acetomethoxy derivative of Calcein

Table 5
Services and cost assessment for screening at the HTSCF in comparison to selected HTS facilities in the north-east

Service	MSKCC	Rockefeller University	Columbia University	ICCB*
Dedicated scientists per project	✓	x	x	x
Assay optimization	✓	x	x	x
Assay validation	✓	x	x	x
Screening	✓	x	✓	x
Confirmation	✓	x	x	x
Data analysis & reporting	✓	x	x	x
Technical support	✓	✓	✓	✓
Chemical library	400K+	169K	100K	200K+
Cost/data point	Low content	\$0.17	\$0.16	0.23
	High content	\$0.29	-	-

* ICCB, Institute for Chemistry and Cell Biology (Harvard University)