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Preclinical Drug Discovery Research and Training at Vanderbilt

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In a recent In Focus piece, the challenges of developing an interdisciplinary curriculum within the Vanderbilt Institute of Chemical Biology (VICB, www.vanderbilt.edu/vicb) for graduate education in chemical biology were discussed (*1*). Equally challenging was the development of a complementary laboratory training experience to expose students to both chemistry and biology techniques required for a research career in translational chemical biology. At the same time, Vanderbilt University was interested in therapeutic drug discovery and the translation of a wealth of basic research findings into potential treatments for unmet medical needs. This new initiative offered an unparalleled opportunity to train students in the multidisciplinary art of preclinical drug discovery and to shepherd a lead compound from high-throughput screens (HTSs) to *in vivo* proof of concept (POC) in relevant animal models. Toward this training goal, Vanderbilt University and the VICB have aggressively recruited senior scientists with proven track records for developing clinical candidates from pharmaceutical giants such as Bristol-Myers Squibb, Merck, and Lilly with expertise in HTS, medicinal chemistry, neuropsychopharmacology, and *in vivo* behavioral pharmacology. Graduate research training is therefore broad and encompasses assay development, screening, synthetic and medicinal chemistry, drug metabolism, cancer biology, electrophysiology, and *in vivo* behavioral pharmacology.

While maintaining an academic mission is paramount, the novel targets and mechanisms of target modulation being pursued by the VICB Program in Drug Discovery offer opportunities for licensing and provide students the ability to truly impact human health by participating in the development of new medicines. At Vanderbilt, students are able to choose from a variety of therapeutic areas for drug discovery such as neuroscience, oncology, ophthalmology, antivirals, and endocrinology to develop novel therapeutics for schizophrenia, Alzheimer's disease, Parkinson's disease, cancer, diabetic retinopathy, HIV, and diabetes. This broad therapeutic focus provides a rich training environment for students because the primary assays, behavioral/POC assays, and compound profiles are distinct for each disease area. The major drug discovery and training focus is on G-protein-coupled receptors (GPCRs), ion channels, and transporter targets, *vide infra*, with allosteric modulation as the preferred mechanism of action; however, opportunities in the areas of allosteric modulation of kinases, inhibition of protein–protein interactions by small molecules, and the development of cell death/viability assays are rapidly increasing.

The first step toward this goal was establishing a state-of-the-art screening facility. Over the past 3 years, Vanderbilt, through the VICB, has invested heavily in developing an infrastructure to support HTS. Fundamental to this effort is the belief that HTS can be an extremely valuable capability in aca-

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demic settings to help investigators discover and develop small molecules for the advancement of basic research and to provide tools to begin validation of new drug discovery targets. The VICB HTS facility (www.vanderbilt.edu/hts) is based on a philosophy that values the ability to automate complex biological assays to allow screening of difficult-to-screen targets. We rarely use automation to increase throughput; rather, we use it to faithfully execute complex tasks with high precision. The VICB HTS facility presently has two automated screening systems that comprise state-of-the-art liquid handling, plate readers, incubators, and other instruments. Both systems are controlled by the Polara 2.3 scheduler running ThermoFisher F3 articulated robotic arms. The systems are designed to allow the facility to support a wide variety of cell-free and cell-based assays ranging from enzyme assays on purified proteins to phenotypic screens on model organisms such as *Caenorhabditis elegans* and zebrafish embryos.

Though not exclusively, the VICB facility focuses on information-rich assay forms primarily in cell-based or organism-based environments. Our main read modes are based either on parallel acquisition of kinetic data derived from two Hamamatsu FDSS kinetic imaging plate readers (Figure 1) or on object-based screening with the BlueShift Isocyte. Both of these readers yield complex, information-rich data sets, and the analysis and storage of such data can be quite challenging. We are working with Hamamatsu, BlueShift, and collaborating investigators to develop methods to extract the highest value out of these instruments by developing new uses and new data analysis methods.

As a screening facility, we understand that our success in discovering small molecule tools is directly linked to our ability to acquire, synthesize, store, and deliver compounds for high throughput screening campaigns.

At present, the VICB HTS facility houses two compound collections. One collection, purchased by Vanderbilt, contains ~160,000 samples acquired mostly from commercial sources. We are continually adding to this collection through new synthesis *via* the VICB chemistry core and the synthetic organic and medicinal chemistry programs. In addition, we are working in-house and with several other academic institutions to acquire natural products collections to further expand the diversity of our collection and hopefully to increase our ability to provide a starting place to develop useful chemical tools. The other collection, which has ~100,000 samples, is from the Small Molecule Repository collected and distributed by Galapagos/DPI for the Molecular Libraries Screening Center Network (MLSCN), discussed below.

We take great care in managing these compound collections to help ensure that the compounds are given every opportunity to reveal their activities. Recently, we have partnered with Nexus Biosystems to acquire an automated compound storage and retrieval system that will allow us to establish best practices in compound storage, including the flexibility to store plates and containers of a variety of sizes in a −20 °C, dry, low-oxygen environment. We are working with Nexus, Labcyte, and ThermoFisher to develop a fully integrated capacity for the rapid, noncontact generation of screen plates, cherry picking, and compounds diluted for the construction of concentration–response curves. We aim to ensure the best chances for our compounds to be in solution, not cross-contaminated with other samples, at the expected concentration, and in the best condition.

For extramural and some intramural investigators, the VICB HTS center works as a member of the 10-site MLSCN. This network of screening centers is a part of the National Institutes of Health (NIH) Roadmap Initiative and is designed to provide investigators worldwide with the ability to access

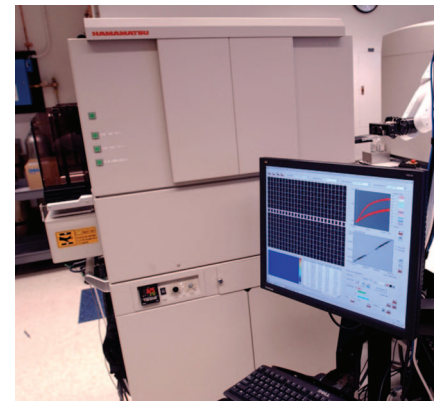

Figure 1. One of the fluorescence drug screening system kinetic imaging plate readers from Hamamatsu used in the VICB HTS facility enabling HTS and routine screening to establish SARs.

HTS and chemistry resources to discover and develop chemical tools. For the MLSCN effort, the VICB facility focuses on GPCRs, ion channels, and transporter targets, whereas other network screening centers have different specializations and capabilities. All data from this network are deposited to a public access database, PubChem. Funding to support HTS is available from NIH through various mechanisms, including assay development grants and resource access grants for obtaining screening resources through the MLSCN. Information about PubChem, the MLSCN, and funding mechanisms can be found at http://mli.nih.gov.

An HTS facility cannot produce a substantial and sustained scientific impact on drug discovery by itself; however, it can be a valuable and enabling part of an early discovery process when the facility and its staff work together as members of multidisciplinary teams that are comprise of investigators with backgrounds in biology, chemistry, pharmacology, behavior, and other relevant areas. This facility allows graduate students to develop cell lines and assays, miniaturize their screens for compatibility with HTS formats, and perform routine screens of new compounds to develop structure–activity

relationships (SARs) to drive medicinal chemistry programs.

After an HTS has been completed, students confer with a medicinal chemist to triage the screening data and prioritize compounds for lead optimization campaigns. Because resources are limited, screening leads are optimized utilizing high-throughput medicinal chemistry techniques in state-of-the-art laboratories. As in the HTS facility, students are exposed to cutting-edge technology and taught classical medicinal chemistry strategies and tactics that go beyond simply improving potency or target selectivity. A single graduate student optimizes the lead compounds from an HTS by employing an iterative analogue library synthesis approach (Figure 2). Solution-phase parallel synthesis (polymer-supported reagents/scavengers, microwave-assisted organic synthesis) and custom mass-directed HPLC purification are used to optimize multiple chemical series in parallel. This technology platform allows a single student to synthesize 24–48 new compounds per chemical series each week for submission to the VICB HTS facility for routine screening as 10 mM DMSO stock solutions in 96-well plates. Delivery days are coordinated with the screening effort; preliminary data are available within 24 h of compound submission so that the next iteration of library synthesis can be initiated. This rapid turnaround of data coupled with expedited chemical synthesis allows for rapid development of SARs and prioritization of chemical series.

Once the SAR has been established in a chemical series, advanced analogues are evaluated in standard drug metabolism and pharmacokinetic (DMPK) assays. First, the students measure the ability of their lead molecules to inhibit the cytochrome P450 (CYP) enzymes and determine plasma protein binding. Compounds with >4% free fraction that do not inhibit CYPs are advanced for further study. Next, compound solubility is measured in standard vehicles,

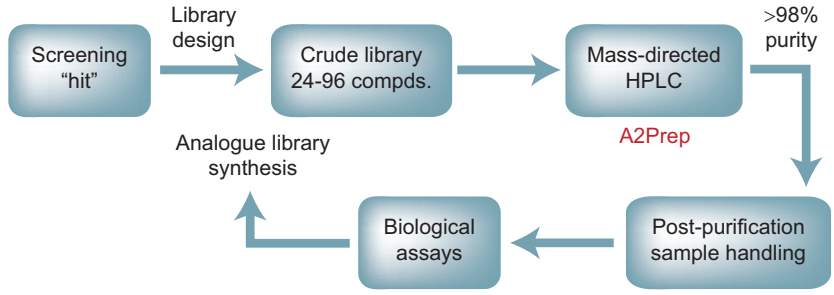

Figure 2. The iterative library synthesis work flow that enables a single graduate student to support a nascent program, develop a SAR, and deliver a POC molecule. Iterations are not driven by potency alone; inputs from DMPK experiments also drive library design. All compounds are purified to analytical purity by mass-directed preparative HPLC.

and then rat pharmacokinetics are evaluated. An ideal POC compound for a novel target/mechanism will possess low to moderate clearance (<20 mL/min/kg) in the rat with >20% oral bioavailability and >2 h half-life so that the compound can be dosed orally for POC studies. Because centrally active molecules are required for central nervous system targets, students determine brain:plasma:cerebrospinal fluid ratios after oral dosing and prioritize compounds with the best plasma:cerebrospinal fluid ratio. At this point, students then submit the compound to a large panel of receptors for a clean ancillary profile to ensure accurate interpretation of *in vivo* POC data. For certain targets, students will collaborate with the radiology department to prepare tritiated analogues for the development of *in vivo* occupancy assays and positron emission tomography tracers to determine plasma occupancy (Occ_{50}) and for use as biomarkers in the clinic.

At this point, students will transition back into a pharmacology laboratory to evaluate their compounds in POC experiments, which vary according to the disease area. Significant resources have been invested for this stage of preclinical drug discovery as well. For oncology, students will evaluate their compounds in caspase-3 assays, 24-h tolerability studies, and finally, mouse tumor xenografts. Within the neuroscience program, students will have the opportunity to conduct extensive biochemical, neurochemical, and/or electrophysiological studies to further elucidate the underlying cellular mechanism(s) of action of their optimized lead compounds, followed by *in vivo* characterization by using preclinical rodent models relevant to their disease area. For all *in vivo* studies, students can take advantage of two different neurobehavioral facilities: one for rat behavioral studies through a collaboration with the VA Tennessee Valley Healthcare System and a second for mouse behavioral work through the Vanderbilt Murine Neurobehavioral Laboratory. Both behavioral cores are located on the Vanderbilt Medical Center campus. Each facility provides extensive behavioral equipment and training expertise for the assessment of novel compounds in a variety of rodent models of neurologic and psychiatric disease states, including chronic pain, drug abuse, anxiety, depression, epilepsy, cognitive impairment, schizophrenia, and motor deficits associated with Parkinson's disease.

To support this new research training program, Vanderbilt competed in a Roadmap competition and was awarded an Interdisciplinary Training in Therapeutic Discovery training grant. Significantly, Vanderbilt was the only institution that was awarded a program grant dedicated to drug

discovery and testing therapeutic hypotheses. The training grant provides formal training for four graduate students and three postdoctoral fellows and each will be co-mentored by two individuals, one with interests in chemical biology and the other with interests in translational medicine. Working together, they will develop a hypothesis for a new therapeutic approach to treat an unmet medical need and then generate a POC molecule (small molecule or antibody) to test the hypotheses *in vitro* and *in vivo*. This new mechanism will make heavy use of VICB cores and will provide direct training for the student or postdoctoral fellow in working as part of a multidisciplinary team to generate a novel agent to treat a disease.

In addition to the training grant, Vanderbilt graduate students engage in VICB drug discovery projects through a number of diverse avenues. Student researchers come from classical chemistry, biology and pharmacology graduate programs as well as the Integrated Graduate Program (IGP) and the Chemical and Physical Biology (CPB) graduate program. Of these, the IGP program is unique and deserves further discussion. Since 1992, graduate students have received a comprehensive educational foundation for a successful career in biomedical research through the Interdisciplinary Graduate Program in the Biological and Biomedical Sciences (IGP). Students entering the IGP take nine months of core coursework and rotate through three laboratories of their choice. The main goal of the IGP year is not to absorb vast quantities of facts, but to learn how to be a creative and analytical thinker who can gain information as needed from the scientific literature. At the end of the IGP year, students select a training program in one of the participating departments or programs, which include biochemistry, biological sciences, cancer biology, cell & developmental biology, human genetics, microbiology & immunology, molecular physiology & biophysics, neuroscience, pathology, pharmacology and drug discovery. One of the greatest benefits of the IGP is the flexibility it offers. New graduate students have nine months to explore their interests in multiple areas before selecting a thesis advisor. The comprehensive nature of the IGP training sometimes leads students to explore departments they hadn't considered previously. Moreover, research faculty often have appointments in more than one department, and students can use the entire IGP year to determine which department most suits their individual interests and career goals. This broad training enables students to fully engage in, and make the most of the laboratory drug discovery experience.

The VICB preclinical drug discovery training program was designed to serve two important functions: to expose students to chemistry and biology techniques required for a research career in translational chemical biology and to translate basic research discoveries into potential therapeutics for unmet medical needs. Students are trained by experienced drug discovery scientists employing state-of-the-art technology across multiple disciplines. They gain experience and exposure to every facet of modern drug discovery and can impact human health during their graduate careers.

REFERENCE

1. Sulikowski, M. M., and Bachmann, B. O. (2006) Designing a curriculum that goes beyond a list of topics, *ACS Chem. Biol. 1*, 489–491.