UM support for NIH Programs in Drug/Probe Discovery and Development

Martha Larsen
HTS Director, CCG
May 2013
NIH Screening Grants

• PAR-12-058 Solicitation of Assays for High Throughput Screening (HTS) to Discover Chemical Probes (R01)
  [link](http://grants.nih.gov/grants/guide/pa-files/PAR-12-058.html)

• PAR-12-059 Solicitation of Assays for High Throughput Screening (HTS) to Discover Chemical Probes (R21)
  [link](http://grants.nih.gov/grants/guide/pa-files/PAR-12-059.html)

• PAR-13-135 High Throughput Screening (HTS) to Discover Chemical Probes (R03) [Aug 6, Dec 12]

• PAR-13-134 High Throughput Screening (HTS) to Discover Chemical Probes (X01) [Aug 6, Dec 12]
  [link](http://grants.nih.gov/grants/guide/pa-files/PAR-13-134.html)
Screening RFA Highlights /Review expectations

• Aimed at generating **probes** (chemical tools or potential leads for drug development)
• Strong Rationale for the target/system to be studied and need for probe/lead (novelty etc.)
• Demonstration that the assay is “screen-ready”
  – S/N, Z’, %CV, reproducibility, pilot results
• Appropriate description of follow-up including secondary assays to remove or identify false positives
• Plan to show utility of probes in cellular/in vivo studies
Grant Details

• **R03**: $100K total, max $50K/year, 2 yrs.
• **R21**: $275K total, max $200K/year, 2 yrs.
• **R01**: no limit, modular budget, $250K*/year, 1-5 yrs. *amts >$250K need detailed budget

• X01 is resource access funding: contact Martha Larsen for more information
Participating NIH Institutes:

- National Institute of Mental Health (NIMH)
- National Cancer Institute (NCI)
- National Eye Institute (NEI)
- National Institute on Aging (NIA)
- National Institute on Alcohol Abuse and Alcoholism (NIAAA)
- National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
- Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
- National Institute on Deafness and Other Communication Disorders (NIDCD)
- National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
- National Institute on Drug Abuse (NIDA)
- National Institute of General Medical Sciences (NIGMS)
- National Institute of Neurological Disorders and Stroke (NINDS)
Screening Grant Tips

• Have a preliminary screen (2-3,000 compounds) done in advance with good S/N, Z’, and CV%
  – If you don’t have the data in hand now, seriously consider waiting for next deadline (Sept/Oct)…but start working with HTS lab now.

• R21 vs R01 vs R03—scope and $
  – If you can easily do the work in the R03 or R31 budget, don’t stretch. Study sections are not dumb.

• Put sufficient attention into the follow-up studies such as hit verification and mechanistic studies as well as how you anticipate probes will be used

• SOA does include commercial analog acquisition and may include mechanism of action studies but not significant Medicinal Chemistry

  PAR-12-060 Solicitation of Validated Hits for the Discovery of in vivo Chemical Probes (R01)

• Check the specific interests of your institute (in SOA)

• Discuss data sharing in your grant
UM Cores/Resources for Drug Discovery Grants

http://cdnm.lsi.umich.edu

- Center for Chemical Genomics (LSI)
- Vahlteich Med Chem Core (COP)
- Pharmacokinetics Lab (COP)
- Other resources at UM
  - Structural Biology (LSI-CSB/Pathology-NMR)
  - Animal Models Cores (Molecular Imaging, Nutritional Metabolism & Obesity Center, Cancer Center Animal Models Core)
  - MICH-R/Phase I Clinic
UM Resources

• Center for Discovery of New Medicines-CDNM
  cdnmlsi.umich.edu
  – Links to UM Core DD Resources
  – Links to NIH Program Announcements

• CCG – mjlarsen@umich.edu santee@umich.edu
  – Screen advice – CCG website FAQs/handouts/staff
  – Compound library sizes & screen estimator (.xls)
  – Budget items for inclusion/estimation
  – Contacts for grant sections and funded examples

• VMCC – sdlarsen@umich.edu showalh@umich.edu
  – Plan for compound selection (triage),
  – Commercial powder acquisition, quality, and analysis
  – In vivo probe development grant assistance
  – Contacts for grant sections and funded examples
HTS Principles

Center for Chemical Genomics
May 2013
Martha Larsen, HTS Director
HTS Grant Outline

1. General Background/Significance
2. HTS Implementation
   a. Assay Selection
   b. Assay Optimization (in home lab)
   c. Assay Adaptation for HTS (in screening center)
   d. Pilot Screen
   e. Hit definition
   f. Screening Library Selection
   g. Full Screen
3. HTS Validation
   a. Hit Follow-Up
   b. Secondary Assays
   c. Chemoinformatic Analysis
   d. Structure Verification
HTS defined

• Assay: Discrete data set from specific set of materials and reagents to determine activity of samples
• Screen: One to many assays performed using same process.
• High-throughput screening (HTS): The process for rapid assessment on the activity of samples from a compound collection, often by running parallel assays in plates of 96, 384, 1536 or 3456-wells
• “high” throughput is a relative term (usually >10⁴)
HTS Assays

Three **requirements** for HTS:

1. Need a target
2. Need to have compounds to test
3. Need to “Keep it simple”
   - Exception in tough screens
   - Use of multiplexed platform
HTS assays: Major classes

Enzymatic - Fluorescence or absorbance

Luminescence

TR-FRET

HCS (cellular localization)

Target Selection

• Check PubChem Bioassay for your target
• Biological information
• Structural information
• Known substances that affect target
• Suitable assay (in vitro or cellular; check PubChem)
• Available reagents, readers & resources
• Counter-screens to assure specificity
• Functional secondary assay available to test “hits” and select “leads”

The more you have, the better your chance of success.
In vitro biochemical assays

• Targets:
  – Enzyme
  – Receptor-ligand
  – Protein-protein interaction
  – Kinase/phosphatase

• Methods we support:
  FP, FI, FRET, TR-FRET, Abs, ALPHA, Bead-based flow, Chemiluminescence, HTRF, Thermal stability

*we discourage ELISA
*we do not support radioactive methods like SPA
Cellular assays

• Targets:
  – Receptor (binding, signaling, internalization)
  – Ion channels
  – Nuclear Receptors
  – Genes, proteins, cofactors
  – Cell processes: cycle, differentiation, fusion, viability, motility
  – Intracellular targets: ER, mitochondrial, lysosomal
  – Molecular translocation or redistribution

• Methods we support:
  Reporter (Abs, FI or lum), FI, ALPHA, BRET, HTRF, FRET, Flow, Imaging
  *we don’t support radioactive
  *calcium flux (FI) is medium throughput (96-well)
Screening Strategy

“Keep it simple…keep it robust”

• Prefer simple protocol
  – Avoid wash steps
  – Need time between steps for multiple plates
  – Ambient incubations, if possible
  – Avoid protocols with lots of additions

• Use inexpensive and available reagents
  – HTS libraries is ~500, 384-well plates
  – Be aware of the cost per well
  – Commercial vs. specialty reagents
  – Recovery of dead volume possible?
  – Reference compounds
  – Calibration standards
  – Stability of reagents
  – calculate reagent needs

(for 500 plates, @15ul/well = 3 Liters)

• Stability of your assay
  – How long before your assay is no longer valid?
  – How long before the last plate reads?

• How long will it take to screen your collection?
  – Miniaturization to high-density plates
  – How many plates/day for your protocol?
  – Calculate time needs

• Assay interference
  – Each compound has its own set of properties (remember to address how you will remove known interfering compounds)

• Compounds
  – Format of compound plates standardized; test concentration is 8-15uM.
Bench vs. HTS lab

We run controls (eg. 32-64 wells for 384-well) on EVERY plate.

Pilot compound screen is 10, 384-well plates.

For Screening:
- Primary assay: n=1
- Retest (confirmation) assay: n=3
- Dose response assay: n=2, 8 doses

Assay Plate

Screening Plate
<table>
<thead>
<tr>
<th>CCG LIBRARY NAME</th>
<th>TYPE</th>
<th>DATE CREATED</th>
<th>COLLECTION</th>
<th>AVAILABLE</th>
<th>SIZE</th>
<th>Screen includes</th>
<th>Number samples</th>
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<tbody>
<tr>
<td>BioFocus NCC</td>
<td>Compounds</td>
<td>3/7/08</td>
<td>NIH collection of FDA drugs</td>
<td>Y</td>
<td>446</td>
<td>N</td>
<td>0</td>
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<td>CMLD</td>
<td>Compounds</td>
<td>8/19/08</td>
<td>Boston and Kansas University Collection</td>
<td>Y</td>
<td>1221</td>
<td>Y</td>
<td>1221</td>
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<tr>
<td>ChemDiv</td>
<td>Compounds</td>
<td>2/3/05</td>
<td>ChemDiv(CCG-15029 to CCG-35028)</td>
<td>Y</td>
<td>20000</td>
<td>Y</td>
<td>20000</td>
</tr>
<tr>
<td>ChemDiv_100K</td>
<td>Compounds</td>
<td>4/16/09</td>
<td>100K ChemDiv Collection</td>
<td>Y</td>
<td>100000</td>
<td>Y</td>
<td>100000</td>
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<tr>
<td>Chembridge 10000</td>
<td>Compounds</td>
<td>10/18/03</td>
<td>Chembridge(CCG-3029 to CCG-15028)</td>
<td>Y</td>
<td>10000</td>
<td>Y</td>
<td>10000</td>
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<tr>
<td>Chembridge 3028</td>
<td>Compounds</td>
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<td>Chembridge(CCG-2001 to CCG-3028)</td>
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<td>3028</td>
<td>Y</td>
<td>3028</td>
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<tr>
<td>Gestwicki Lab Extracts</td>
<td>Substances</td>
<td>9/26/07</td>
<td>Natural Extracts from Gestwicki Lab</td>
<td>Y</td>
<td>724</td>
<td>N</td>
<td>0</td>
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<tr>
<td>Kinase_Inhibitors</td>
<td>Compounds</td>
<td>12/4/07</td>
<td>Annotated and analogs</td>
<td>Y</td>
<td>323</td>
<td>Y</td>
<td>323</td>
</tr>
<tr>
<td>MS Spectrum 2000</td>
<td>Compounds</td>
<td>1/18/06</td>
<td>MicroSource(CCG-38339 to -40338)</td>
<td>Y</td>
<td>2000</td>
<td>N</td>
<td>0</td>
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<tr>
<td>Maybridge_HF</td>
<td>Compounds</td>
<td>7/18/06</td>
<td>Maybridge(CCG-40575 to CCG-56574)</td>
<td>Y</td>
<td>16000</td>
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<tr>
<td>NCI_HTS</td>
<td>Compounds</td>
<td>12/6/05</td>
<td>Screening collection</td>
<td>Y</td>
<td>3149</td>
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<tr>
<td>Natural Extracts</td>
<td>Substances</td>
<td>4/19/05</td>
<td>Sherman Lab Extracts &amp; Fractions</td>
<td>Y</td>
<td>32291</td>
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<td>Focused Libraries</td>
<td>Compounds</td>
<td>3/6/2012</td>
<td>Autophagy, Wnt Pathway, Epigenetics, Protein Kinase, Protease, REDOX, Cannabinoid, NPs</td>
<td>Y</td>
<td>1037</td>
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<tr>
<td>Fragments</td>
<td>Compounds</td>
<td>9/10/2012</td>
<td>Asinex fragment library</td>
<td>?</td>
<td>2668</td>
<td>N</td>
<td>0</td>
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</tbody>
</table>

* Some compounds are in more than one library

- All of these compounds are listed in PubChem and any CCG data can be published or shared.
- We have demos of MScreen and online tutorials on features and navigation.
- We have over 20 million compounds from vendor libraries accessible via search in MScreen.
- Our diversity collection is different (~5% overlap) from MLSCN collection.
- Fragment library can be used for Thermal stability, NMR, SPR, etc.
Z’ (Z-factor): statistical parameter used to evaluate and validate HTS assays

\[
Z’ = 1 - \frac{(3 \times \text{SD}_{\text{min}} + 3 \times \text{SD}_{\text{max}})}{|\text{Avg}_{\text{min}} - \text{Avg}_{\text{max}}|}
\]

It’s about the S/N **AND** the variability, some assays can be less than recommended S/N > 3
MScreen data of pilot screen

![Graph showing MScreen data](image)

<table>
<thead>
<tr>
<th>ASSAYID</th>
<th>Z_FACTOR</th>
<th>ASSAYDATE</th>
<th>INVESTIGATOR</th>
<th>TARGETNAME</th>
<th>METHODNAME</th>
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<tbody>
<tr>
<td>711</td>
<td>0.47</td>
<td>2008-02-12</td>
<td>Neubig</td>
<td>RGS4 (-Cys)</td>
<td>Accuri_Hypercyt</td>
</tr>
</tbody>
</table>
“hits” “leads” “drugs” “probes”

- **Hit** is what you get by testing in HTS (aka “active”)
- **Lead** is what you get after confirming HTS assay “hit” in another “secondary” assay
- **Candidate** is what you have after your “lead” satisfies the medical chemists, pharmacologists and pharmacokinetists (ADME, animal studies, toxicology)
- **Drugs** are candidates that have activity in humans (INDA, NDA)
- MLP defines a **probe** as a compound that can be useful as a research tool. “It does not have to work in animals but ideally it will work in cells”
Probe

• **Small molecule** identified via biochemical assays exhibiting potencies of \(<\textbf{500nM}\) and, more ideally in the 100nM range. Those identified in cell based assays are expected to exhibit potencies at a level of \(<\textbf{1uM}\), however in certain instances potencies in the 10uM may be acceptable.

• **Availability:** The probe molecule should be accessible (and soluble) in amounts to allow advanced studies (15-20mg), and protocols for its preparation or isolation should be made available.

• **SAR, mode of action:** (e.g. evidence of binding to target, characterization of mechanism of action) and awareness of **selectivity** against relevant and/ or related targets is expected.
HTS success is a “Good” “Hit”

• Activity is “target mediated”
  – Not false positive (i.e. inactive in confirmation and/or counterscreen)
  – Not due to an artifact of the assay itself
  – Active in secondary and tertiary screens

• Potency
  – Ideal: < 1 uM
  – Good: 1-10 uM
  – Acceptable: 10-30 uM

• Physicochemical Properties
  – No toxic groups
  – Good predicted chemical stability (i.e. not likely to have interfered with assay)
  – Good permeability potential (yes this IS important for probes, too!)
    • MW < 500
    • Total H-bond donors < 5
    • H-bond acceptors < 10
    • LogP < 5
    Polar surface area (tPSA) < 140
    No strong acids (e.g. SO$_3$H), diacids or quaternary ammonium salts

• Potential for progression from Hit to Lead
  – SAR (wide range of activity induced by small changes in structure)
  – Ideally more than two chemical series (backup)
  – Free from conflicting intellectual property (this doesn’t apply to probe research)
Estimated Attrition Rates from Screen to Hit

Screening Collection

Primary Screen
Repeat Primary Screen
Dose Response

 Obtain New Sample
Repeat Dose Response
Secondary, Selectivity Screens

“Leads”

---

Same conditions, collection sample

Same conditions

Different conditions

Increasing Confidence

---

150,000 samples
0.1 – 2% “actives” 1500
~30% repeat 500
~50% titrate 250
~50% available 125
~50% titrate 60 – 70
10-40% “hits” 5 – 30

Overall: Only ~2% of “actives” will become “hits”
# Suggested items to include in budget for screening grants

Sample Budget for a Biochemical Assay Screening Campaign  
150K compound screen in 384-well plates at 15 μL assay volume

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCG staff* at 5%</td>
<td>$3600</td>
</tr>
<tr>
<td>PI Lab - Research Personnel Costs</td>
<td>Budget for 2-4 months</td>
</tr>
<tr>
<td>CCG Assay Charges**</td>
<td>$25,000</td>
</tr>
<tr>
<td>Reagent Costs, e.g. Proteins, detection reagents</td>
<td>$ varies</td>
</tr>
<tr>
<td>(~3.5L of assay reagents at their final concentration)</td>
<td>$ varies</td>
</tr>
<tr>
<td>Purchase of New Library Compounds (optional)</td>
<td>$ varies</td>
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<tr>
<td>Fresh Powder for re-testing “Hits”</td>
<td>$5000</td>
</tr>
<tr>
<td>Follow-up dose response on powders</td>
<td>$2500</td>
</tr>
<tr>
<td>Counterscreens &amp; Secondary screening (eg. Cell-based)</td>
<td>$ varies</td>
</tr>
<tr>
<td>Chemoinformatics and Medicinal Chemistry Consultation</td>
<td>$7500</td>
</tr>
</tbody>
</table>
## Suggested items to include in budget for screening grants

**Sample Budget of Mammalian Cell-based Screening Campaign**

150K compound screen in 384-well plate at 40ul assay volume

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
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<tbody>
<tr>
<td>CCG staff* at 5%</td>
<td>$3600</td>
</tr>
<tr>
<td>PI Lab-Research Personnel Costs</td>
<td>Budget for 3-6 months</td>
</tr>
<tr>
<td>CCG Assay Charges**</td>
<td>$36,000</td>
</tr>
<tr>
<td>Reagent (eg. luminescent gene reporter)</td>
<td>$10,000</td>
</tr>
<tr>
<td>Media (~10L for assay plates)</td>
<td>$ varies</td>
</tr>
<tr>
<td>Fresh Powder for re-testing “hits”</td>
<td>$5000</td>
</tr>
<tr>
<td>Follow-up dose response on powders)</td>
<td>$7000</td>
</tr>
<tr>
<td>Counterscreens &amp; Secondary screening (eg. Phenotypic)</td>
<td>$5000</td>
</tr>
<tr>
<td>Chemoinformatics and Medicinal Chemistry Consultation</td>
<td>$7500</td>
</tr>
</tbody>
</table>
Request Project at CCG website
Collaborating with the VMCC on Screening Grants PAR-12-058 and -059

Scott D. Larsen, PhD, Director
Vahlteich Medicinal Chemistry Core (VMCC)
College of Pharmacy
Role of VMCC in Early Drug Discovery at UM

• **Lead Discovery**: Center for Chemical Genomics (CCG)
  – Assay development
  – High Throughput Screening
  – Confirmation of actives

• **Lead Optimization**: Vahlteich Medicinal Chemistry Core (VMCC)
  – Follow-up of HTS actives
  – Analog design and synthesis
  – Patenting advice and assistance

• **Lead Development**:
  – Pharmacokinetics (UM PK Core, Pharm Sci Dept)
    • Metabolic stability, metabolite ID
    • In vivo drug levels, distribution and clearance rates
  – In Vivo Target Validation (PI labs)
    • Tolerability
    • Efficacy in animal model of disease
  – Partner or out-license
How Can VMCC Help?

• Prepare HTS follow-up plan
  – Hit triage process
    • Med chem “inspection” for potential chemical reactivity or toxicity
    • Analysis of structure-activity relationships from HTS and confirmation screens
  – Initial SAR expansion
    • Selection of similar compounds in CCG
    • Selection of commercial analogs
  – Final lead selection (with specific criteria) for probe use or further development

• Collaborate on grant preparation to further develop leads (e.g. PAR-12-060)
  – Suggestion: use the “Technical Prerequisites” from PAR-12-60 as your “goals” for PAR-12-058/-059
Contact Info

• Scott Larsen (sdlarsen@umich.edu)
• Hollis Showalter (showalh@umich.edu)
• Paul Kirchhoff (pkirchho@umich.edu)
• Visit website: www.umvmcc.org
Contact Info

- HTS: Martha Larsen (mjlarsen@umich.edu)
- NPE: David Sherman (davidhs@umich.edu)
- Med Chem: Scott Larsen (sdlarsen@umich.edu)
- Chemoinformatics: Paul Kirchhoff (pkirchho@umich.edu)
- PK: Duxin Sun (duxins@umich.edu)
**Project Title:** High Throughput Screening (HTS) to Discover Graft-Versus-Host Disease Inhibitors

**SRG Action:** Impact Score: 17  Percentile: 2

**Next Steps:** Visit http://grants.nih.gov/grants/next_steps.htm

**Human Subjects:** 10-No human subjects involved

**Animal Subjects:** 30-Vertebrate animals involved - no SRG concerns noted

<table>
<thead>
<tr>
<th>Project Year</th>
<th>Direct Costs Requested</th>
<th>Estimated Total Cost</th>
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<tbody>
<tr>
<td>1</td>
<td>250,000</td>
<td>388,750</td>
</tr>
<tr>
<td>2</td>
<td>250,000</td>
<td>388,750</td>
</tr>
<tr>
<td>3</td>
<td>250,000</td>
<td>388,750</td>
</tr>
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</table>

ST2 target for small molecules high-throughput screening (HTS). Preliminary data includes a pilot screen (using an Alphascreen assay that gives z'- factors ≥ 0.7) resulting in 17 hits from 2450 compounds of known biological activity comprising of the Spectrum Collection and the NIH Clinical Collection.

Chemical inhibitors targeting the ST2/IL33 interaction will be identified and then validated in vitro human assays and in vivo murine models of GVHD systems to assess ST2 as the therapeutic target.

- The University of Michigan is a very supportive environment that provides all necessary facilities to carry out proposed HTS studies, the SAR studies and the in vitro/in vivo secondary screens for functional activity of the leads identified in the HTS.

Drs. Larsen and Larsen (director of HTS in the core laboratory of the Center for Chemical Genomics and co-director of the University of Michigan Vahlteich Medicinal Chemistry Core (VMCC) bring core access and expertise for the HTS component of the project.
CCG_Assay Formats

- Biochemical Assays (Isolated proteins or enzymes)
  - Absorbance (Abs)
  - Fluorescence polarization (FP)
  - Fluorescence/Forster Resonance Energy Transfer (FRET)
  - Time-Resolved FRET (TR-FRET)
  - Fluorogenic substrates (FI)
  - Scintillation proximity (SPA) *radioactive*
  - ALPHA (Amplified Luminescent Proximity Homogeneous Assay)-also in cell-based
  - Bioluminescence, Chemiluminescence
  - Thermal Shift
  - Label-free (SPR, Res Wavelength Grating, ITC, Biolayer interferometry) coming soon!

- Cell-based Assays (Microbes, Mammalian recombinant or primary cell lines)
  - Luciferase reporter-genes (luminescent)
  - Intra-molecular calcium flux
  - Cell fusion
  - Intracellular fluorescent substrates (FI)
  - Bioluminescence resonance energy transfer (BRET)-also in biochemical assays
  - Membrane translocation
  - Homegenous TR Fluorescence (HTRF)-also in biochemical (eg kinase) assays
  - Label-free coming soon!

- Other Assays
  - Whole animal (eg. Zebrafish)
  - Flow cytometry (FCM and FCPIA)
  - High Content Imaging (HCI/HCS)
CCG_Assay Validation

- Robustness
  - Use “real” screening conditions
  - Identify sources of errors
- Validation parameters
  - S:B, CV, Z-factor
  - Plate to plate variability
  - Assay to assay variability
- Validation criteria
  - Number of plates
  - Batch and lot of reagents
  - Calibration controls on each plate
  - Spiked ref compounds (inhibitors/activators)
  - Multiple days assay
  - Power of observation
PAR-12-060

• Solicitation of Validated Hits for the Discovery of \textit{in vivo} Chemical Probes (R01)
  
  – \url{http://grants.nih.gov/grants-guide/pa-060.html}

• “This program creates an opportunity for integrated research in biology and chemistry on structure-activity relationships (SAR) of novel compounds through an iterative and parallel optimization process, which ultimately allows the researchers to take a holistic approach and \textit{make rapid progress toward successful development of in vivo chemical probes}”
What PAR-12-060 covers...

- *In vitro* cellular and tissue activities (potency, selectivity, specificity, etc.);
- *In vitro* structural, physicochemical, and biochemical properties (solubility, stability, membrane permeability, protein binding, microsome stability, metabolite identification, CYP inhibition, etc.);
- *In vivo* pharmacokinetics (PK) with absorption, distribution, metabolism, excretion (ADME) and toxicity;
- *In vivo* efficacy
Other Info for PAR-12-060

- 3-year R01 (up to $500K/yr)
- Participating institutes
  - National Institute of Mental Health (NIMH)
  - National Cancer Institute (NCI)
  - National Eye Institute (NEI)
  - National Institute on Aging (NIA)
  - National Institute on Alcohol Abuse and Alcoholism (NIAAA)
  - National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
  - Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
  - National Institute on Deafness and Other Communication Disorders (NIDCD)
  - National Institute on Drug Abuse (NIDA)
  - National Institute of General Medical Sciences (NIGMS)
  - National Institute of Neurological Disorders and Stroke (NINDS)
- Not participating:
  - National Heart, Lung, and Blood Institute (NHLBI)
  - National Human Genome Research Institute (NHGRI)
  - National Institute of Allergy and Infectious Diseases (NIAID)
  - National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
- Expires Sept 2014
What You Need First...

• Lead compound/series
  – must elicit a reproducible response in at least two assay types and also elicit a dose-response over a hundred-fold concentration range;
  – must be analytically validated in terms of integrity and purity (e.g., use of resynthesized powder sample of high purity in the preliminary assays);
  – must demonstrate adequate potency;
  – must possess a tractable starting point of chemical optimization with no obvious major chemical liabilities.

• Assays
  – Solid testing funnel in place including animal model
How Can VMCC Help?

• Prepare med chem plan
  – Improving potency/selectivity
  – Optimizing physical properties for in vivo efficacy
    • Solubility
    • Metabolic stability
    • Permeability
  – Scaling up synthesis of selected cmpds for in vivo assays
    – Recommend contacting Duxin Sun for inclusion of PK studies
• Consult on design of testing funnel
**Example Probe R01 (funded)**

- **Title:** Novel Probes for Studying Treatment of CNS-based Lysosomal Storage Diseases (LSDs)
- **Background:** Lead inhibitor of Glucosylceramide Synthase (GlcCerS) successful at treating peripheral LSD (Gaucher Type 1) but does not penetrate CNS. New probe from our lab reduces GlcCer in brain, but is cleared rapidly in vivo. Can we develop more suitable probes for studying long term effects of GlcCerS inhibition in the CNS?
Specific Aims

• **Prepare new potential probes with physical properties predictive of good PK and CNS exposure**
  – Optimize properties favoring blood-brain barrier (BBB) permeability
    • Lower tPSA, MW and amine basicity, and continue to explore conformational restriction
    • Evaluate all new analogs for GlcCer synthase inhibition
    • Evaluate all active analogs for MDR1 recognition *in vitro* and construct pharmacophore model to guide design
    • Evaluate lead analogs for recognition by other efflux transporters
  – Optimize metabolic stability
    • Identify sites of metabolism of CCG-203586 by mouse liver microsomes
    • Design and synthesize analogs that block sites of metabolism
    • Evaluate all active compounds for metabolism by mouse liver microsomes

• **Identify brain-penetrant probes for *in vivo* studies**
  – Perform PK studies in normal mice
  – Determine brain/plasma levels in selected disease model(s)

• **Determine effects of acute and chronic inhibition of GlcCer synthase in the CNS**
  – Profile glycosphingolipid changes over time in wild-type mice
  – Evaluate probes in mouse models of neuronopathic LSDs (Sandhoff, GM1 gangliosidosis)
Probe Testing Funnel

**Broken MDCK cell for GlcCer synth (100 cmpds)**

- IC$_{50}$ < 100 nM

- **WT- and MDR1-MDCKII cell for GlcCer synth (50 cmpds)**
- Rhod 123 uptake into MDR1-MDCKII cells (50 cmpds)

- WT IC$_{50}$ < 50 nM
- MDR1 IC$_{50}$/WT IC$_{50}$ < 2
- Rhod 123 < 200% ctrl

**Stability to mouse liver microsomes (25 cmpds)**

- T$_{1/2}$ > 30 min

**C57 Mouse PK (10 cmpds)**

- CL < 40 ml/min/kg
- C$_{max}$ > 10x IC$_{50}$
- Brain/plasma > 0.5

**Disease model mouse PK (5 cmpds)**

- C$_{max}$ > 10x IC$_{50}$
- Brain/plasma > 0.5

**Acute mouse models (2-3 cmpds)**

**Chronic mouse model (1-2 cmpds)**