Identification of Repurposed Drugs for Chordoma Therapy.

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Division of Pre-Clinical Innovation
National Center for Advancing Translational Sciences
National Institutes of Health
**NIH Chemical Genomics Center**

- **Founded in 2004**
  - National Center for Advancing Translational Sciences (NCATS)
  - >100 staff: Biologists, Chemists, Informatics and Engineers

- **Robotic HTS facility**

- **Mission**
  - Development of chemical probes for novel biology
  - Novel targets, rare/neglected diseases
  - New technologies/paradigms for assay development, screening, informatics, chemistry

- **Collaborations**
  - >200 investigators worldwide
    - 60% NIH extramural
    - 25% NIH intramural
    - 15% Foundations, Research Consortia, Pharma/Biotech
Steps in the drug development process

1. Create testing system (aka, “assay”)
2. Test >100,000 chemicals for activity on target
3. Make modifications to active chemicals to make suitable for human use
4. Test in animals for safety, effectiveness
5. Test in humans for safety, effectiveness
Two approaches to therapeutics for rare and neglected diseases

1-2 years?

>400,000 compounds, 15 yrs

3500 drugs
The NCGC Pharmaceutical Collection

### Informatics sources for NPC
- US FDA: Orange Book, OTC, NDC, Green Book, Drugs at FDA
- Britain NHS
- EMEA
- Health Canada
- Japan NHI

### Physical sources for NPC
- Procurement from >70 suppliers worldwide
- In-house purification of APIs from marketed forms
- Synthesis

### Drug Source

<table>
<thead>
<tr>
<th>Drug Source</th>
<th>In house</th>
<th>Procurement in process</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>US FDA*</td>
<td>1635</td>
<td>182</td>
<td>1817</td>
</tr>
<tr>
<td>UK/EU/Canada/Japan</td>
<td>756</td>
<td>177</td>
<td>933</td>
</tr>
<tr>
<td>Total Approved</td>
<td>2391</td>
<td>359</td>
<td>2750</td>
</tr>
<tr>
<td>Investigational</td>
<td>928</td>
<td>3953</td>
<td>4881</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3319</strong></td>
<td><strong>4312</strong></td>
<td><strong>7631</strong></td>
</tr>
</tbody>
</table>

* These counts include approved veterinary drugs

### Drug plate composition

- Investigational: 28%
- US FDA: 23%
- Canada/UK/EU/Japan: 49%

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* NIH National Center for Advancing Translational Sciences

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* NCATS NCATS
The NCGC Pharmaceutical Collection: A Comprehensive Resource of Clinically Approved Drugs Enabling Repurposing and Chemical Genomics

Ruili Huang,* Noel Southall,* Yuhong Wang, Adam Yasgar, Paul Shinn, Ajit Jadhav, Dac-Trung Nguyen, Christopher P. Austin

Small-molecule compounds approved for use as drugs may be “repurposed” for new indications and studied to determine the mechanisms of their beneficial and adverse effects. A comprehensive collection of all small-molecule drugs approved for human use would be invaluable for systematic repurposing across human diseases, particularly for rare and neglected diseases, for which the cost and time required for development of a new chemical entity are often prohibitive. Previous efforts to build such a comprehensive collection have been limited by the complexities, redundancies, and semantic inconsistencies of drug naming within and among regulatory agencies worldwide; a lack of clear conceptualization of what constitutes a drug; and a lack of access to physical samples. We report here the creation of a definitive, complete, and nonredundant list of all approved molecular entities as a freely available electronic resource and a physical collection of small molecules amenable to high-throughput screening.
Chordoma Screening Project

• Cell lines
  ➢ Chordoma cell lines screened: U-CH1 and U-CH2B
    ▪ Counter-screen: CCL4b

• Assay readout
  ➢ Luminescent Cell Viability Assay
  ➢ Measurement of the number of viable cells based on quantitation of ATP, an indicator of metabolic activity
  ➢ Luminescent signal proportional to amount of ATP present

• 2816 approved drugs screened each at multiple concentrations (0.5 nM to 46 uM) for 48 h

ATP + Luciferin + O₂ → Oxyluciferin + AMP + PPI + CO₂ + Light

Luciferase
Quantitative High-Throughput Screening (qHTS)

- Compounds assayed at multiple concentrations
  - ≥ 7 concentrations
- Assay concentration ranges over 4 logs (high:~ 100 μM)
- Miniaturized assay volumes 2-6 μL in 1536-well plate
- Informatics pipeline for data processing, curve fitting & classification, extraction of SAR
- Generates *pharmacological actives* rather than statistical “hits”
  - Dramatically increases reliability
  - Dramatically reduces false positives and false negatives
Compound Selectivity of NPC_2816 Library in Cell Types

- inactive, 2219, 78.8%
- other, 510, 18.1%
- uch selective, 7, 0.2%
- uch1 selective, 4, 0.1%
- uch2b selective, 47, 1.7%
- active in all, 29, 1.0%

Legend:
- active in all
- uch selective
- uch1 selective
- uch2b selective
- inactive
- other
Active Compounds with Selectivity

- Active in all three cell lines
- Selective for UCH1 and 2B
- Selective for UCH1
- Selective for UCH2B
Cheery-pick confirmation

- Total: 60 compounds
  - UCH selective compounds (inactive in CCL4B): 7
  - UCH1 selective compounds: 4
  - UCH2B selective compounds (IC50 <20 µM): 32
  - Interesting compounds active in all three cell lines (known anticancer compounds; potentially interesting mechanism; more selective for UCH1 or UCH2; not apparent promiscuous toxic compounds): 4
  - Known RTK inhibitors and other kinase inhibitors available in house: 13
- Retested in Cell Titer Glo assay: UCH1, UCH2B, CCL4B
- Confirmation rate: 96%
Tested 60 selected compounds in UCH1 and UCH2 cell lines
• 5, 16, 24 and 48 hr in caspase 3/7 and cell viability assays
• With few exceptions, compounds that killed cells also activated caspase-3/7 at some point, and vice versa
Powder Confirmation in Primary Patient Cells

- Re-ordered and tested 35 compounds
  - C24, C25 and C32
  - UCH1
  - UCH2b
Bortezomib treatment in the presence of one concentration (IC20) of Rubitecan, Camptothecin, Doxorubicin, or Topotecan
Summary and Next Step

• Repurposed FDA approved drugs including combination treatment can be used for Chordoma treatment

• Manuscript in revision

• Collaboration for testing in animal models
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