Optimized Experimental and Analytical Tools for Reproducible Drug-Response Studies

CB399 – March, 2017
Motivation: Making better drugs and identifying patients who will benefit

http://www.cancer.gov/
### Magic bullet therapies (Die Zauberkugeln)

**Paul Ehrlich**
(1854 -1915)

<table>
<thead>
<tr>
<th>Mutation Activity</th>
<th>Disease</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Her2/ErbB2</td>
<td>Kinase (RTK)</td>
<td>Breast Cancer</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>Kinase (Y)</td>
<td>CML</td>
</tr>
<tr>
<td>EGFR^{L858R}</td>
<td>Kinase (RTK)</td>
<td>Lung Cancer (NSLC)</td>
</tr>
<tr>
<td>BRAF^{V600E}</td>
<td>Kinase (S/T)</td>
<td>Melanoma</td>
</tr>
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<td>EML4/ALK</td>
<td>Kinase (RTK)</td>
<td>Lung Cancer (NSLC)</td>
</tr>
</tbody>
</table>
Dose-response relationships: the basic figure of merit for a drug

Anti-cancer Drugs

Drug Dose ($\log_{10}$)

Fraction Surviving

Better drug

Worse drug

$IC_{50}$
Dose-response on a large scale

http://lincs.hms.harvard.edu/fallahi-sichani-nchembio-2013/
Why is this data valuable: drug pharmacogenomics

http://www.cancerrxgene.org/
Rethinking what really varies in dose-response relationships

\[ y = E_{\text{inf}} + \left( \frac{E_0 - E_{\text{inf}}}{1 + \left( \frac{D}{E_{C_{50}}} \right)^{HS}} \right) \]
What does the literature say?

Theory

Potency

Efficacy

Literature

<table>
<thead>
<tr>
<th>HS</th>
<th>$E_{\text{max}}$</th>
<th>$IC_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(50)</td>
<td>(117)</td>
<td>(15,467)</td>
</tr>
</tbody>
</table>

PubMed Citations
Extensive variation in potency and efficacy

**Potency**

**Efficacy**

Hafner/Heiser et al., Scientific Data, in preparation
Fractional response (to TRAIL) is a stable property of cell populations

TRAIL → (survivors) Recover ~3 days TRAIL → (survivors)
Cell-to-cell variability and its impact on dose response at a population level

Sources of Variation

Genome:
- mutations
- epigenetic changes
- expression levels
- etc.

Environment:
- growth factors
- cell-cell interaction
- nutrients
- etc.

Proteome:
- protein levels
- protein localization
- protein modification
- complex formation

Typical Stochastic Fluctuation

top 5% / bottom 5% = 5x

CV=0.5

Theory

Population Response
Single Cell Response

Real Data

Geldanamycin

GSK2126458

log_{10} [Drug (M)]

Relative viability

Drug Concentration

Response

Cell A

Cell B

E_{max} = 0.5

HS <<1
Application to a targeted therapy \((\text{BRAF}^{\text{V600E/K}} \text{ melanoma})\)
Understanding residual disease: drug response in single BRAF^{V600E} melanoma cells

Control

Live cell imaging using cell-cycle reporter

+Vemurafenib -

H2B-Venus $\rightarrow$ tracks nuclear morphology
mCherry-geminin $\rightarrow$ tracks cell cycle progression
Live-cell imaging to monitor single-cell phenotypes

- **DMSO (Control)**
  - COLO858 individual cells over time (h): 12, 24, 36, 48, 60, 72, 84
  - Phases: Cycling, Apoptotic

- **Vemurafenib (1 μM)**
  - COLO858 individual cells over time (h): 12, 24, 36, 48, 60, 72, 84
  - Phases: Slowly-cycling, Non-cycling, Apoptotic

**Resistant:** Neural crest (NGFR+)

**Sensitive:** Melanoma (MITF+)

BRAF inhibition
Single-cell effects and the time-dependence of response

- **Starting/Reset Cells**
  - Vemurafenib
  - ~7 days reset time
  - 80-90% kill

- **Survivors**
  - 1 day
  - BET Inhibitors

- **Slow-growing de-differentiated**
  - Vemurafenib

- **Transient Resistance**
  - Vemurafenib

- **Sustained Resistance**
  - Vemurafenib
Typical dose-response curves for anti-cancer drugs (LINCS-optimized protocols)