Developing Predictive Bioactivity Signatures of Carcinogenesis Using ToxCast HTS Data

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Predicting Toxicity Will Not Be Easy

Chemical

- Poor Bioavailability
- Downstream Indirect effects
  - XME
  - PPAR
  - MoA 2
  - MoA 3
  - Enhancer
  - Suppressor
  - Direct Chemical Targets
  - Rat Liver Lesions
Key Challenges Of Pathway Profiling

• Find the Toxicity Pathways
  • Hepato vs developmental neurotoxicity

• Obtain HTS Assays for Them
  • Including metabolic capability

• Screen Chemical Libraries
  • Coverage of p-chem properties

• Link Results to in vivo Effects
  • Gold standard and dosimetry
ToxCast™ Background

- Research program of EPA’s National Center for Computational Toxicology
- Addresses chemical screening and prioritization needs for pesticidal inerts, anti-microbials, CCLs, HPVs and MPVs
- Comprehensive use of HTS technologies
- Coordinated with NTP and NHGRI/NCGC via Tox21
- Committed to stakeholder involvement and public release of data
  - Chemical Prioritization Community of Practice
  - NCCT website- http://www.epa.gov/ncct/
The ToxCast_320

309 Unique Structures

Replicates for QC

291 Pesticide Actives
9 Industrial Chemicals
8 Metabolites

56/73 Proposed Tier 1 EDSP

53 of 80 with DNTs

122 in IRIS

14 HPV
11 HPV Challenge

Classes with > 3 chemicals

- Acetylcholine esterase inhibitors
- conazole fungicides
- Sodium channel modulators
- pyrethroid ester insecticides
- organothiophosphate acaricides
- dinitroaniline herbicides
- pyridine herbicides
- thiocarbamate herbicides
- imidazolinone herbicides
- organophosphate insecticides
- phenyl organothiophosphate insecticides
- aliphatic organothiophosphate insecticides
- amide herbicides
- aromatic fungicides
- chloroacetanilide herbicides
- chlorotriazine herbicides
- growth inhibitors
- organophosphate acaricides
- oxime carbamate insecticides
- phenylurea herbicides
- pyrethroid ester acaricides
- strobilurin fungicides
- unclassified acaricides
- unclassified herbicides

Misc MOA classes with 3 or fewer representatives
ToxRefDB

- Relational phenotypic/toxicity database
- Provides in vivo anchor for ToxCast predictions

- Three study types
  - Chronic/Cancer Rat and Mouse (Martin, et al, EHP 2008)
  - Rat multigenerational Reproduction (Martin, et al, 2009)
  - Rat & Rabbit Developmental Toxicity (Knudsen, et al, 2009)

- Two types of synthesis
  - Supervised (common individual phenotypes)
  - Unsupervised (machine based clustering of phenotype patterns)
ToxRefDB Endpoint Coverage

**CHRONIC/CANCER (CHR)**
Martin et al. (2008) Environ Hlth Persp
doi:10.1289/ehp.0800074

**MULTIGENERATION REPRODUCTIVE (MGR)**
Martin et al. (2009) Toxicol Sci
doi: 10.1093/toxsci/kfp080

**PRENATAL DEVELOPMENTAL (DEV)**
Knudsen et al. (2009) Reprod Toxicol
doi: 10.1016/j.reprotox.2009.03.016

**SOURCE: Matt Martin, NCCT, 2009**
Rat Chronic Bioassay Results

Multigeneration Study Results

Martin, et al, 2009
ToxRefDB in Predictive Modeling

**STRENGTHS**

- Source data from >2,000 guideline studies
- Puts >$2B worth of legacy data into a computable form
- *in vivo* database anchoring HTS *in vitro* assays
- Enables comparison of endpoint incidence between species
- Searchable database will be public ([www.epa.gov/ncct/toxrefdb/](http://www.epa.gov/ncct/toxrefdb/))

**LIMITATIONS**

- Endpoints aggregated as independent features
- Data largely qualitative (LELs, LOAELS)
- Not all ToxCast™ chemicals represented in ToxRefDB
- Not all ToxRefDB chemicals represented in ToxCast™
- Species dimorphism may link to biology or study design
- Limited mode of action information available in source DERs
ToxCast Assays

Biochemical Assays

- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter

- Assay formats
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

Cellular Assays

- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney

- Primary cells
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells

- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes

- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

467 Endpoints
Confidence Builders: Some Expected Results…

- Estrogen receptor (ER)
  - Bisphenol A, Methoxychlor, HPTE
- Androgen Receptor (AR)
  - Vinclozolin, Linuron, Prochloraz
- PPAR
  - PFOA, PFOS, Diethylhexyl Phthalate, Lactofen
- Mitochondrial Poisons
  - Azoxystrobin, Fluoxastrobin, Pyraclostrobin
- Acetylcholinesterase Inhibition
  - Multiple organophosphorus pesticides
Confidence Builders (2): Multiple Assays and Technologies per Target
Confidence Builders (3): Pathway Based Analysis

Biologically Multiplexed Activity Profiling (BioMAP)

Multiplex Transcription Reporter Assay

Cell-based HTS Assays

Cell-free HTS Assays

High Content Cell Imaging Assays
Data Analysis:
What is a hit?
Biochemical HTS from Novascreen

- hCYP 2C9
- hERα
- rAdrRa2B
- hLynA Activator
- hM1
- hKATPase
qHTS from the NCGC on NRs

ERα

PPARγ

log Concentration [M]

Acitivities [%]

NCGC00164420-01
NCGC00093991-03
NCGC00164230-01
NCGC00022570-07

NCGC00090965-03
NCGC00164033-01
NCGC00023462-04
NCGC00025156-10
NCGC000161666-02
NCGC00090749-04
**Attagene: cis and trans Assays**

*trans*: ERα

*cis*: ERE

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**Bisphenol A**

**HPTE**
CellzDirect: Data Examples

**CYP1A1-AhR**

- CYP1A1 (9hr) - EC50: 0.2002
- CYP1A2 (9hr) - EC50: 0.1746
- CYP1A1 (24hr) - EC50: 0.8615
- CYP1A2 (24hr) - EC50: 0.9368
- CYP1A1 (48hr) - EC50: 0.6212
- CYP1A2 (48hr) - EC50: 0.1125

**HMGC22-PPARα**

- HMGC22 (6hr) - EC50: 14.22
- HMGC22 (24hr) - EC50: 4.113
- HMGC22 (48hr) - EC50: 8.509

**CYP2B6-CAR**

- CYP2B6 (6hr) - EC50: 0.0856
- CYP2B6 (24hr) - EC50: 0.05418
- CYP2B6 (48hr) - EC50: 0.04320

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**CYP1A1-AhR**

- Log Concentration (μM)
- Fold Induction / Control vs. Log Concentration (μM)
- Data Points: CYP1A1 (9hr), CYP1A2 (9hr), CYP1A1 (24hr), CYP1A2 (24hr), CYP1A1 (48hr), CYP1A2 (48hr)

**HMGC22-PPARα**

- Log Concentration (μM)
- Fold Induction / Control vs. Log Concentration (μM)
- Data Points: HMGC22 (6hr), HMGC22 (24hr), HMGC22 (48hr)

**CYP2B6-CAR**

- Log Concentration (μM)
- Fold Induction / Control vs. Log Concentration (μM)
- Data Points: CYP2B6 (6hr), CYP2B6 (24hr), CYP2B6 (48hr)
Metabolic Activity from Solidus

![Graphs showing metabolic activity from Solidus](image-url)
ToxCast Phase I Assay Hits
(n=624 measurements)

- Cell Free HTS
- Multiplexed TF
- Human BioMap
- HCS
- qNPAs
- XMEs
- Impedance
- Genotoxicity

828 Assay-Chemical Pairs had AC50s of less than 1µM

Judson et al, EHP, submitted
Minimum Human Pathways in ToxCast Phase I

Judson et al, EHP, submitted

Office of Research and Development
National Center for Computational Toxicology
“Hits” per Chemical
As a Function of AC50/LEC Cutoff

9 Chemicals have at least 20 hits at an AC50 of <30µM

Judson et al, EHP, submitted
“Hit” Distribution for Chemical Classes Against 33 Minimal Pathways (at least 10 chemicals per class)

Judson et al, EHP, submitted
Activity of Conazoles Against Minimal Pathway Set

Judson et al, EHP, submitted
Rat Liver Histopathology from Chronic Bioassays

- **No Pathology**: 68
- **Proliferative Lesions**: 37
- **Pre-neoplastic Lesions**: 21
- **Neoplastic Lesions**: 37

Source: Judson et al, submitted
Rat Liver Tumor Correlations

Fisher’s Exact test, p<0.01

Judson, et al, submitted
Gene Networks Associated with Progression of Rat Liver Tumor Endpoints

Judson et al, EHP, submitted
Some Challenges Faced or to be Faced

- Organizing the chemical library
- Quality control of the chemical library
  - Acceptable purity, stability
- Defining concentration response ranges to the assayed
- Definition/Calculation of a hit
  - Minimum fold change; minimum r-squared; limit on Hill function
- Assay performance
  - Replicates, artifacts
- Sufficient coverage of biological pathways
  - Including those that represent tissue level processes
- Incorporation of metabolic competency
- Establishment of target prediction
  - Pathway perturbation
  - Rodent bioassay data
  - Rodent mechanistic studies
  - Human effects
- Sufficient representation of positives to predict against
## Prioritization Product Timeline

<table>
<thead>
<tr>
<th>Phase</th>
<th>Number of Chemicals</th>
<th>Chemical Criteria</th>
<th>Purpose</th>
<th>Number of Assays</th>
<th>Cost per Chemical</th>
<th>Target Date</th>
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<tbody>
<tr>
<td>Ia</td>
<td>320</td>
<td>Data Rich (pesticides)</td>
<td>Signature Development</td>
<td>552</td>
<td>$20k</td>
<td>FY07-09</td>
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<td>Ib</td>
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<td>Pilot</td>
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<td>Data Rich Chemicals</td>
<td>Validation</td>
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<td>~$20 -25k</td>
<td>FY09-11</td>
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<td>&gt;100</td>
<td>Known Human Toxicants</td>
<td>Extrapolation</td>
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<td>~$20 -25k</td>
<td>FY09-11</td>
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<tr>
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<td>Expanded Structure and Use Diversity</td>
<td>Extension</td>
<td>&gt;400</td>
<td>~$20 -25k</td>
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<td>&gt;12</td>
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<td>PMN</td>
<td>&gt;200</td>
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<td>FY10-11</td>
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<td>Prediction and Prioritization</td>
<td>&gt;300</td>
<td>~$15 -20K</td>
<td>FY11 -12</td>
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**FY07** | **FY08** | **FY09** | **FY10** | **FY11** | **FY12**

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**Proof of Concept: ToxCast**

- Verification/Extension
- Reduce to Practice

**Tox21**
Phase II Plans

• Done in conjunction with Tox21 10k Library
  – Subset of 700 will seed Phase II

• Chemical Diversity
  – More food use pesticides (~100-200)
  – Failed pharmaceuticals (preclinical and clinical, ~100-150)
  – “Green” chemicals
  – HPV Categories
  – Liver toxicants (~150)
  – OECD Molecular Screening Group nominations

• Evaluation of Phase I Assays

• Addition of new assays via competitive procurements

• Timing
  – Chemical procurement completed 4thQ FY09
  – Launch of Assays, 1st Q FY10
  – Results Available early FY11