What is Translation?

*Translation* is the process of turning observations in the laboratory and clinic into interventions that improve the health of individuals and the public - from diagnostics and therapeutics to medical procedures and behavioral changes.
What is Translational Science?

Translational Science is the field of investigation focused on understanding the scientific and operational principles underlying each step of the translational process.

NCATS studies translation as a scientific and organizational problem.
To catalyze the generation of innovative methods and technologies that will enhance the development, testing and implementation of diagnostics and therapeutics across a wide range of human diseases and conditions.
Some of the *scientific* translational problems on NCATS’ to-do list...

- Predictive toxicology
- Predictive efficacy
- Derisking undruggable targets/untreatable diseases
- Data interoperability
- Biomarker qualification process
- Clinical trial networks
- Patient recruitment
- Electronic Health Records for research
- Harmonized IRBs
- Clinical diagnostic criteria
- Clinical outcome criteria (e.g., PROs)
- Adaptive clinical trial designs
- Shortening time of intervention adoption
- Methods to better measure impact on health (or lack of)
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NCATS Scientific Initiatives

• **Clinical Translational Science**
  » Clinical and Translational Science Awards
  » Rare Disease Clinical Research Network
  » New Therapeutic Uses program

• **Preclinical Translational Science**
  » NIH Chemical Genomics Center
  » Therapeutics for Rare and Neglected Diseases program
  » Bridging Interventional Development Gaps program

• **Re-engineering Translational Sciences**
  » Toxicology in the 21st Century
  » Tissue Chip program
  » Office of Rare Diseases Research
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  - Office of Rare Diseases Research
Toxicity is a common reason for drug development failure

Preclinical (21%) + Clinical (12%) Tox = 33% of all failures

A Grand Challenge: Predicting Toxicity

- Complex Cellular and Tissue Dose HTS
- Toxicity
- Tissues
- Cellular Networks
- Cell Changes
- Molecular Pathways
- Cell-Based HTS
- Model Organism MTS
- Virtual Tissues

Exposure:
- Tissue Dose
- Molecular Targets
- Biochemical HTS

Model Organism MTS

Gene

Toxicology Technology Development

The Tox21 Program
Tox21 Goals

• Identify patterns of compound-induced biological response in order to:
  » characterize toxicity/disease pathways
  » facilitate cross-species extrapolation
  » model low-dose extrapolation

• Prioritize compounds for more extensive toxicological evaluation

• Develop predictive models for biological response in humans
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<th>Area of Expertise</th>
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<th>EPA</th>
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Tox21 10K Compound Library

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- **EPA (3726)**
- **NTP (3194)**
- **NCGC (3526)**

- 88 single-sourced cmpds in duplicate on each plate
- 2255 replicate substances (GSIDs) across 3 inventories

**unique substances**
**unique solution IDs**
**total number of test cmpd wells**

Compound identity and structures available at [http://www.epa.gov/ncct/dsstox/sdf_tox21s.html](http://www.epa.gov/ncct/dsstox/sdf_tox21s.html)
Tox21 Screening Process

Validation
- Positive controls
- Time course
- Signal to background

Miniaturization
- Cell density per well
- Positive controls
- Signal to background ≥ 3
- CV <10%

CV (coefficient of variation) = \[\frac{\text{standard deviation (SD) of compound area}}{\text{median of compound area}}\]

Z factor = \[1 - \frac{3 \times (\text{SD of compound area} + \text{SD of basal})}{\text{median of compound area} - \text{median of basal}}\]

Attene-Ramos et al., 2013, Drug Discovery Today 18:716-723
Quantitative High-Throughput Screening (qHTS)

- Conventional screening done at one concentration
  - Not appropriate for toxicity testing – “dose makes the poison”
- qHTS tests compounds assayed at multiple concentrations
  - For Tox21, 14 concentrations over 4 logs (high: ~ 100 uM)
- Miniaturized assay volumes 2-8 uL in 1536-well plate
- Informatics pipeline for data processing, curve fitting & classification, extraction of SAR
- Generates toxicological actives rather than statistical “hits”
  - Dramatically increases reliability
  - Dramatically reduces false positives and false negatives

Inglese et al., Proc Natl Acad Sci 103:11473, 2006
Tox21 Robotic Screening System
Tox21 Informatics Analysis Process

Plate reads → Normalize → Correct

% Activity

Log[Compound] vs. Activity

Tox21 Data Repository

Tox21 internal Data sharing

Public Data release

PubChem

CEBS

AcTOR

NIH National Center for Advancing Translational Sciences
Tox21 Phase III

• Focused on increased pathway coverage, high content imaging assays, and high throughput gene expression platforms using
  » cells capable of xenobiotic metabolism
  » ES/iPSC derived differentiated cell populations (e.g., cardiomyocytes, neurocytes, hepatocytes)

• Integration of metabolite prediction models into hazard prediction models

• Secondary screens needed to bridge HTS to in vivo toxicology

• Expanded utilization of lower organisms (zebrafish, C. elegans)

Targeted Assays

• High Content screening
  ✓ Hoechst: Cell loss & nuclear size
  ✓ DHE: Oxidative stress/ROS
  ✓ p53: DNA damage
  ✓ pH2A.X: Genotoxicity
  ✓ JC-10: Mitochondrial damage (MMP)
  ✓ Caspase 3: Apoptosis
  ✓ Lipitox: Steatosis & Phospholipidosis
  ✓ Reactive metabolites/ROS: GSH depletion

• Receptor Activation via Induction of gene expression
  ✓ AhR, CAR, PXR, PPARα, FXR

• Necrosis
  ✓ miR-122 leakage or LDH leakage
Tox21 Focused on Secondary Screening Needed to Bridge HTS to in vivo Toxicology

- qHTS
  - ~10,000 cpds

- In vitro secondary assays
  - ~100 cpds

- Gene Expression

- HCS

- Test in rats to predict human toxicity
  - 5-10 cpds
A working hypothesis

1. Any pathway important to normal function/physiology has the potential, when disrupted, to cause pathophysiology - i.e., toxicity

2. To reliably predict potential toxicity of a chemical, its activity on every pathway operant in mammalian (human, rodent) cells must be characterized

3. To allow this characterization, a complete/nonredundant list of all pathways operant in mammalian cells must be enumerated

4. A set of experimental assays, each of which covers ≥1 pathway in network space, could reliably characterize compound activities across pathway space with a desired degree of certainty
The NCGC (NIH Chemical Genomics Center) BioPlanet™

- Hosts universe of pathways
  - Focus on human pathways (~2000 unique)
- All pathway annotations from manually curated, public sources
  - Integrates pathways from >10 different data sources
  - e.g. KEGG, WikiPathways, Reactome, Science Signaling
- Annotates pathways by source, species, biological function/process, disease/toxicity relevance, assay availability
- Easy visualization, browsing, analysis of pathways
- Facilitates pathway assay selection/prioritization for Tox21
- Web version in process for public release
The Universe of Pathways

Detailed view of a pathway

Pathways

Gene information
Pathways with available assays - Tox21, ToxCast, NCGC
Web-based version in development
BioPlanet™ Applications

• Assay selection/prioritization for Tox21
  ➢ Toxicity pathways?
  ➢ Disease pathways?
  ➢ Assay availability?
  ➢ Maximize pathway coverage?

• Future developments
  ➢ Link compound activity data
  ➢ Incorporate other data forms: sequence data, gene/protein expression data, etc.?
  ➢ Other species: rat, mouse, etc.
  ➢ Organize assays according to pathways/diseases/toxicity endpoints
Tissue Chip for Drug Screening Program

• Goal
  ➢ Develop organoids on chips to screen for compound toxicity, efficacy
    ▪ Liver, heart, lung, other cell types
    ▪ Integrate platform systems
    ▪ Designed for multiple different readouts

• NIH, DARPA contributing ~$70M each over 5 years
  ➢ NCATS and DARPA independently manage, fund separately but highly coordinated program
  ➢ FDA provides regulatory science guidance

• Awards announced in 2012
  ➢ Supporting the best ideas in engineering, biology, and toxicology
GOAL: Develop an in vitro platform that uses human tissues to evaluate the efficacy, safety and toxicity of promising therapies.

Tissue Chip Program

- All ten human physiological systems will be functionally represented by human tissue constructs:
  - Circulatory
  - Endocrine
  - Gastrointestinal
  - Immune
  - Integumentary
  - Musculoskeletal
  - Nervous
  - Reproductive
  - Respiratory
  - Urinary

- Physiologically relevant, genetically diverse, and pathologically meaningful.
- Modular, reconfigurable platform.
- Tissue viability for at least 4 weeks.
- Community-wide access.
Tissue Chips from Common Building Blocks

Scaffold
- purified ECM
- synthetic polymers
- composites

Cells
- stem/progenitor
- differentiated
- mixed cell types

Structure
- porosity
- topography
- stiffness

Spatial/Temporal Patterning
- cytokine gradients
- controlled release

Perfusion
- embedded channels
- vascularization

Bioreactors
- optimized culture conditions
- biomechanical properties
- blood mimetics

Computational Design
- systems integration
- multi-scale modeling
- simulation
- feedback

Host Response
- generalized inflammation
- specific immunity

Innervation
- controlled release
- optimized culture conditions
- biomechanical properties
- blood mimetics

Functional Readout
- real-time, label-free, non-destructive sensing
- imaging

Innervation
- signal propagation
- coordinated response
Engineered Cardiac Muscular Thin Films

(A) Fabricate Substrate and Seed myocytes

(B) Cut out shapes

(C) Dissolve sacrificial layer peel off unwanted film

(D) Film bends up as myocytes contract

Data provided by Dr. Kit Parker, Wyss Institute
3-D biomimetic liver sinusoid construct

Sentinel cells: a subpopulation of hepatocytes, stellate and Kupffer cells that stably express biosensors to monitor key cell functions.

• Cytochrome C released from mitochondria
• Exposed to 10µM Nefazadone
• Time-lapse of 16 hours
Body-on-a-Chip?

In vivo Correlation
- Absorption
- Distribution
- Metabolism
- Excretion
- Conc(t)
- Effect(t)
- Toxicity(t)
- Rare toxicities

Read outs
- Human biology
- Tissue/organ structure
- Cell histology
- Cell viability
- Mechanical properties
- Electrical properties
- Signaling pathways
- Cell metabolism
- Protein synthesis
- Gene expression
- Enzyme activities
- Ion channel properties
Program Leads at NCATS

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