The National Center for Advancing Translational Sciences

Validation of In Vitro Microphysiological Systems and the Tox21 Program

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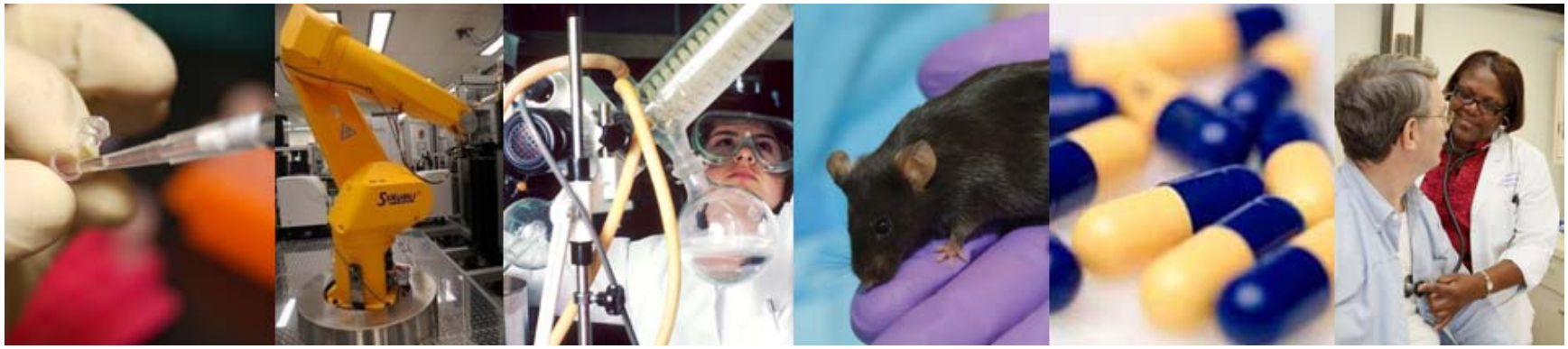
AMERICAN INSTITUTE FOR MEDICAL AND BIOLOGICAL ENGINEERING (AIMBE) / NIH FOURTH WORKSHOP

MARCH 7, 2014

NCATS

NIH National Center for Advancing Translational Sciences

NCATS Mission

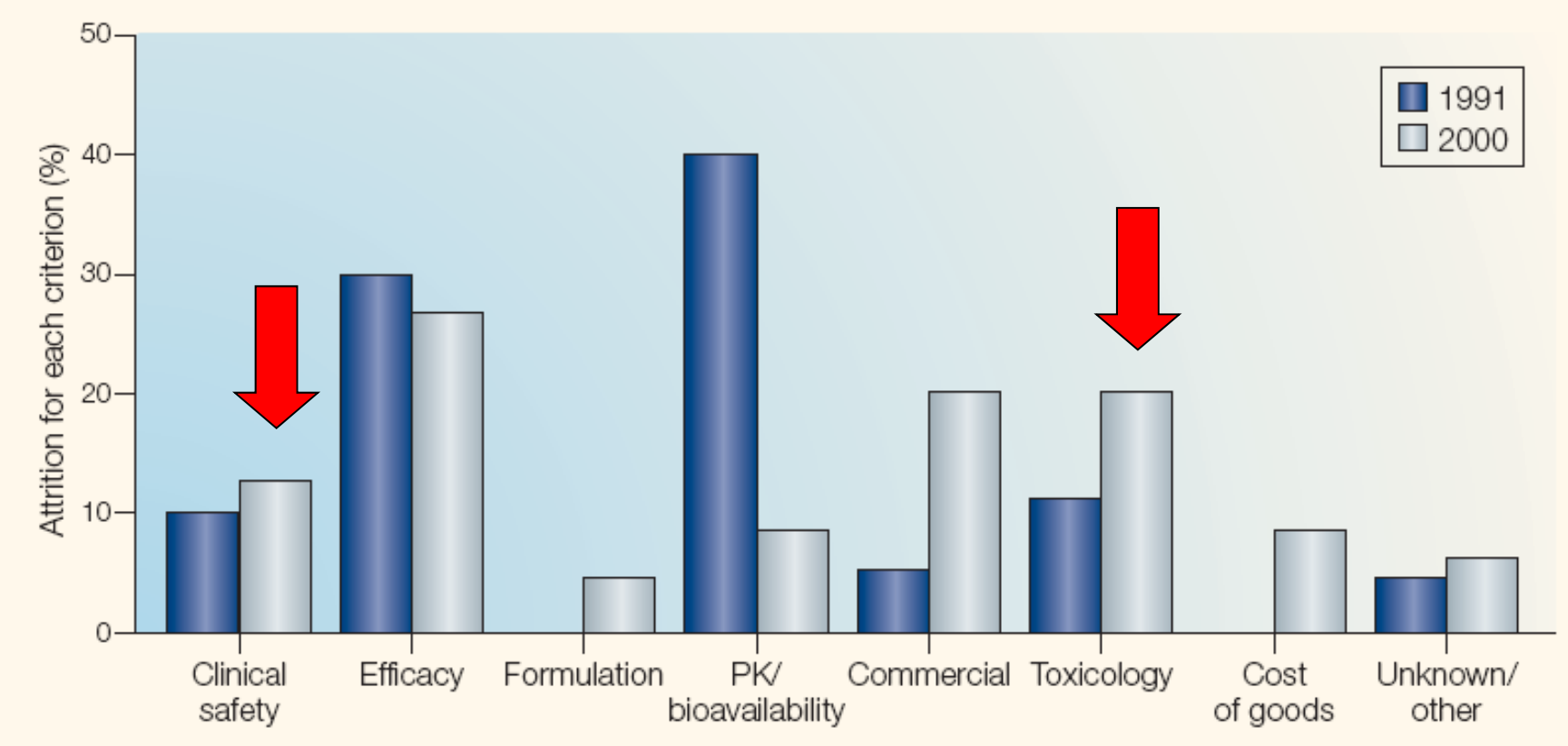


To catalyze the generation of innovative methods and technologies that will enhance the development, testing and implementation of diagnostics and therapeutics across a wide range of human diseases and conditions.

NCATS Advisory Council Subcommittees

- Medical Technologies
  - Frank L. Douglas
  - Paul Yock
- Patient Engagement
  - Margaret Anderson
  - Myrl Weinberg
- Interactions with Biotech/Pharma/VC
  - Freda Lewis-Hall
  - Ankit Mahadevia

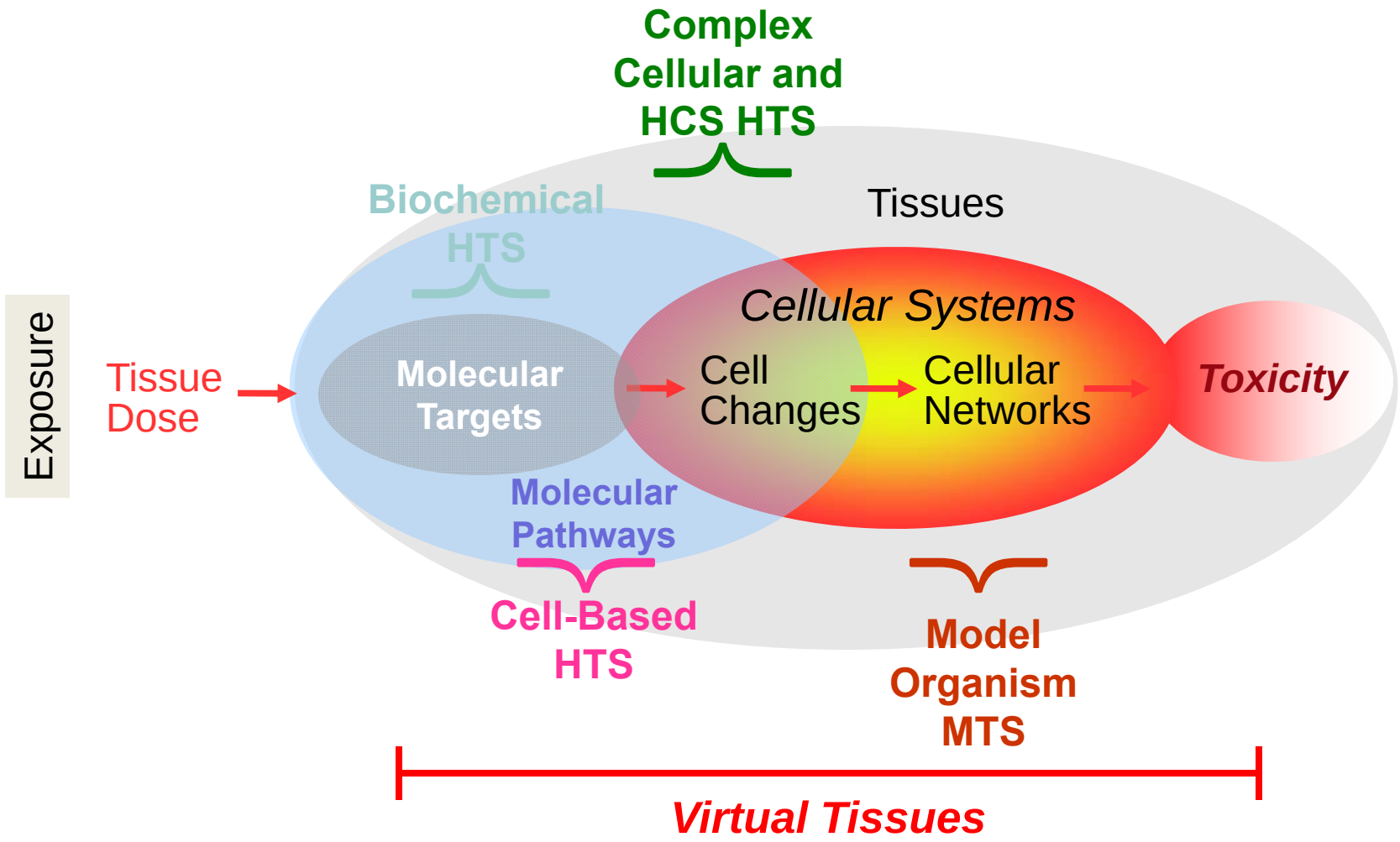
Toxicity is a common reason for drug development failure



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

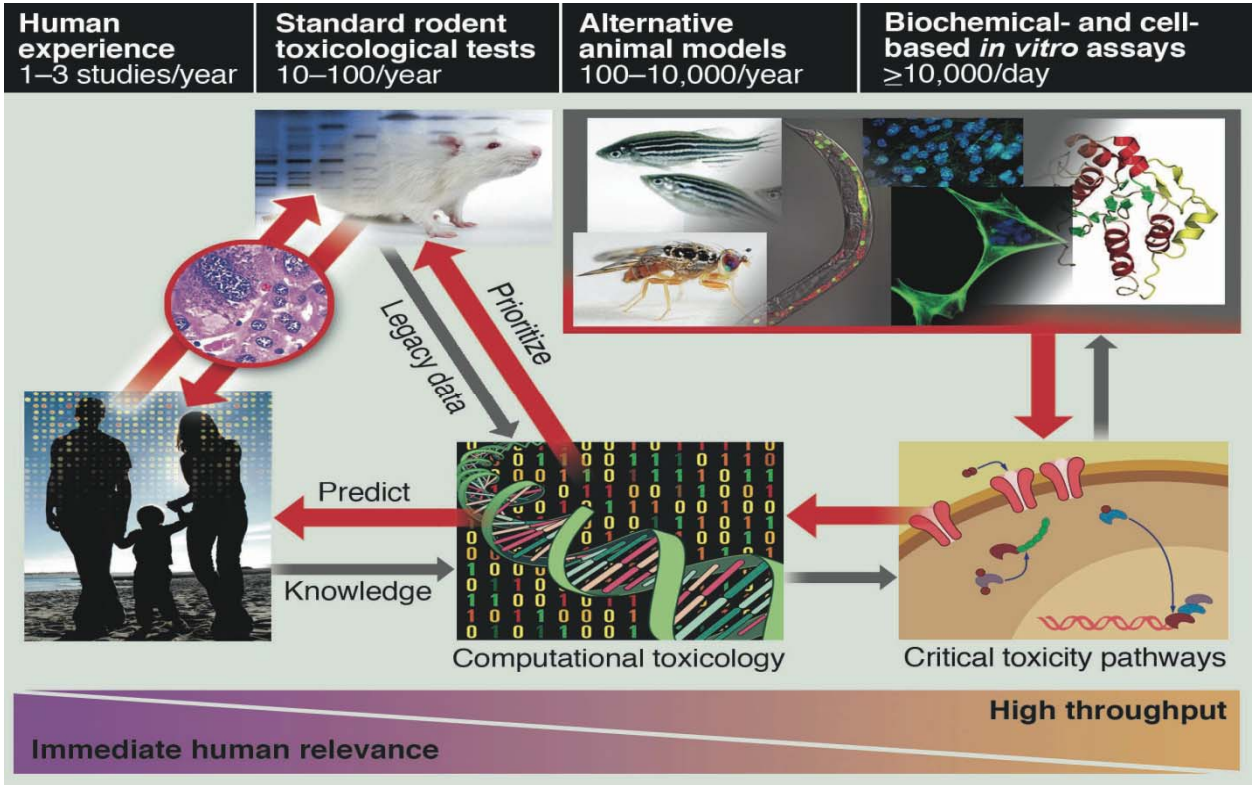
Kola and Landis, *Nature Reviews Drug Discovery* 3, 711-716, 2004.

A Grand Challenge: Predicting Toxicity



Why do we need to prioritize compounds for testing?

- There are over 80,000 chemicals in commerce, the majority with little to no toxicological data
- Problem cannot be solved using laboratory animal testing alone



Collins, FS, Gray, GM, Bucher, JR (2008) Transforming Environmental Health Protection. *Science* 319:906-7

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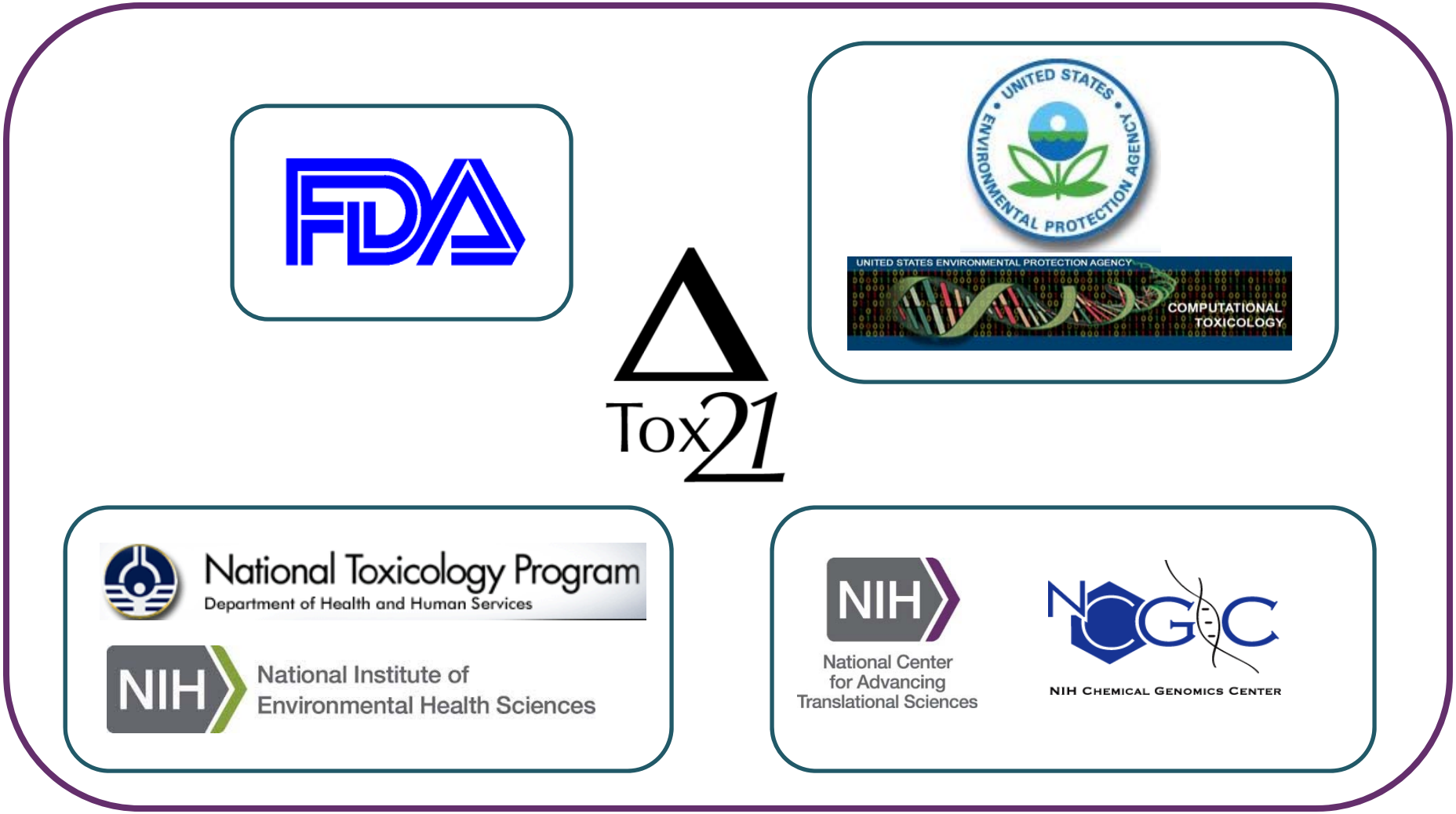
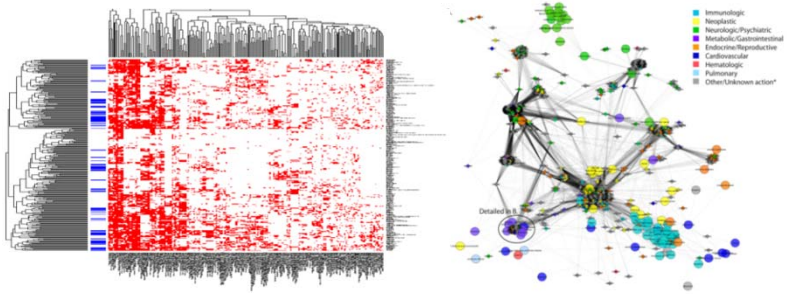
Toxicology Technology Development

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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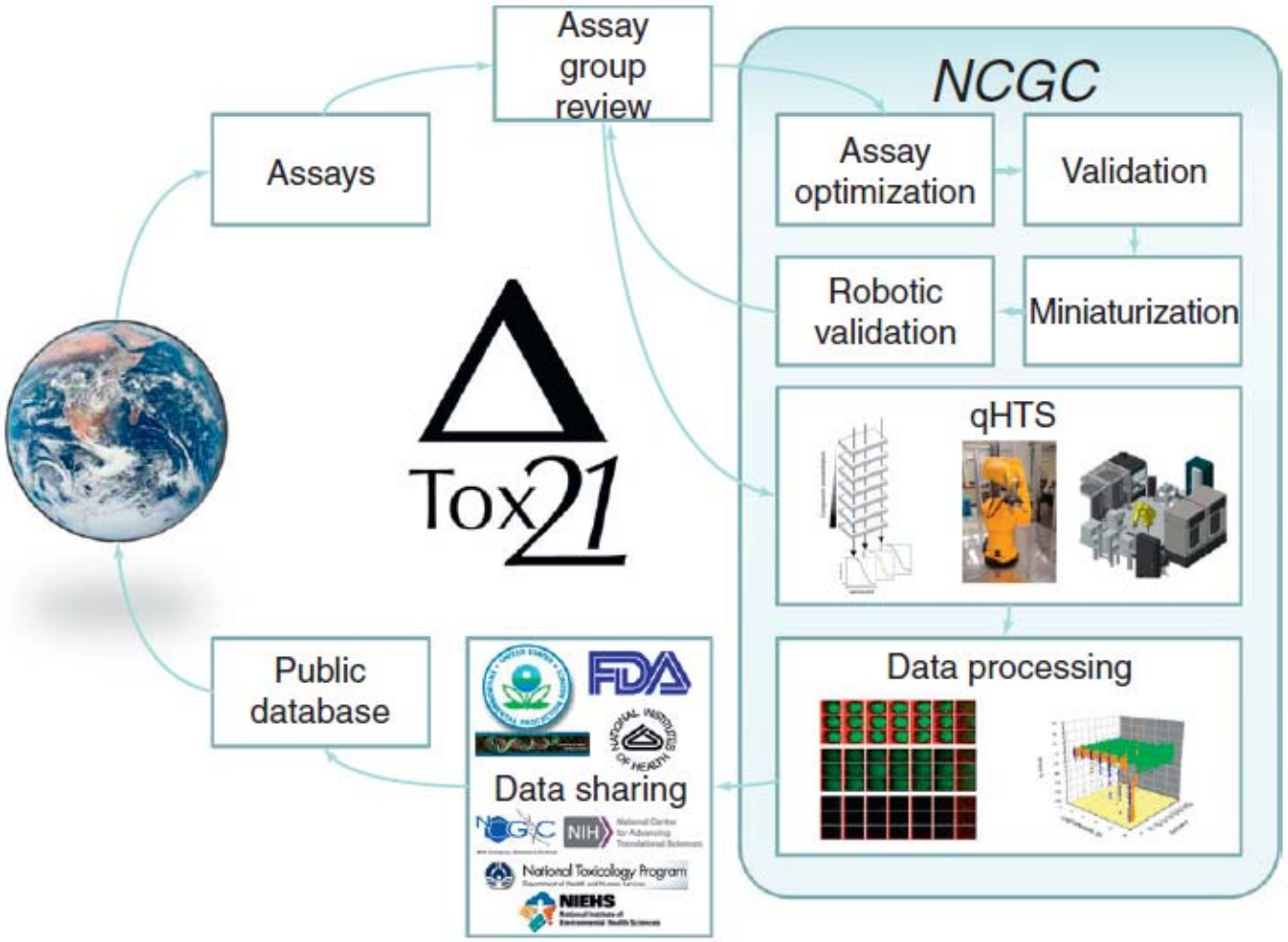
Tox21 Cross-Agency Team

- NCATS
  - Menghang Xia
  - Srilatha Sakamuru
  - Jean Zhao
  - Sampada Shahane
  - Nicole Miller
  - Bonnie Goodwin
  - Amy Hsu
  - Tongan Zhao
  - Yuhong Wang
  - Dac-Trung Nguyen
  - Paul Shinn
  - Sam Michael
  - David Gerhold
  - David Kuo
  - Zhi-Bin Tong
  - Bill Leister
  - Anton Simeonov
- NTP/NIEHS
  - Keith Shockley
  - Jennifer Fostel
  - Kristine Witt
  - Cynthia Smith
  - Suramya Waidyanatha
  - Mike DeVito
  - Raymond Tice
  - John Bucher
- EPA
  - Richard Judson
  - Keith Houck
  - Ann Richard
  - Kevin Crofton
  - David Dix
  - Rusty Thomas
  - Bob Kavlock
- FDA
  - Weida Tong
  - James Weaver
  - Kevin Gaido
  - Donna Mendrick
  - Suzanne Fitzpatrick

Area of Expertise	NIEHS/NTP	NCATS	EPA	FDA
Lab Animal Toxicology	✓		✓	✓
Human Toxicology/Exposure Assessment	✓		✓	✓
Ultra High Throughput Screening		✓		
Low to Mid Throughput Assays	✓	✓	✓	✓
Stem Cell Assay Development	✓	✓	✓	✓
Epigenetic Assays	✓	✓		
Engineered Tissue Models	✓	✓	✓	✓
'Omic Based Systems	✓	✓	✓	✓
Lower Organism Models	✓		✓	✓
Genetic Variability in Response	✓	✓		
Databases & Informatic Tools	✓	✓	✓	✓
Validation Experience	✓	✓	✓	✓

Tox21 Screening Process



Validation

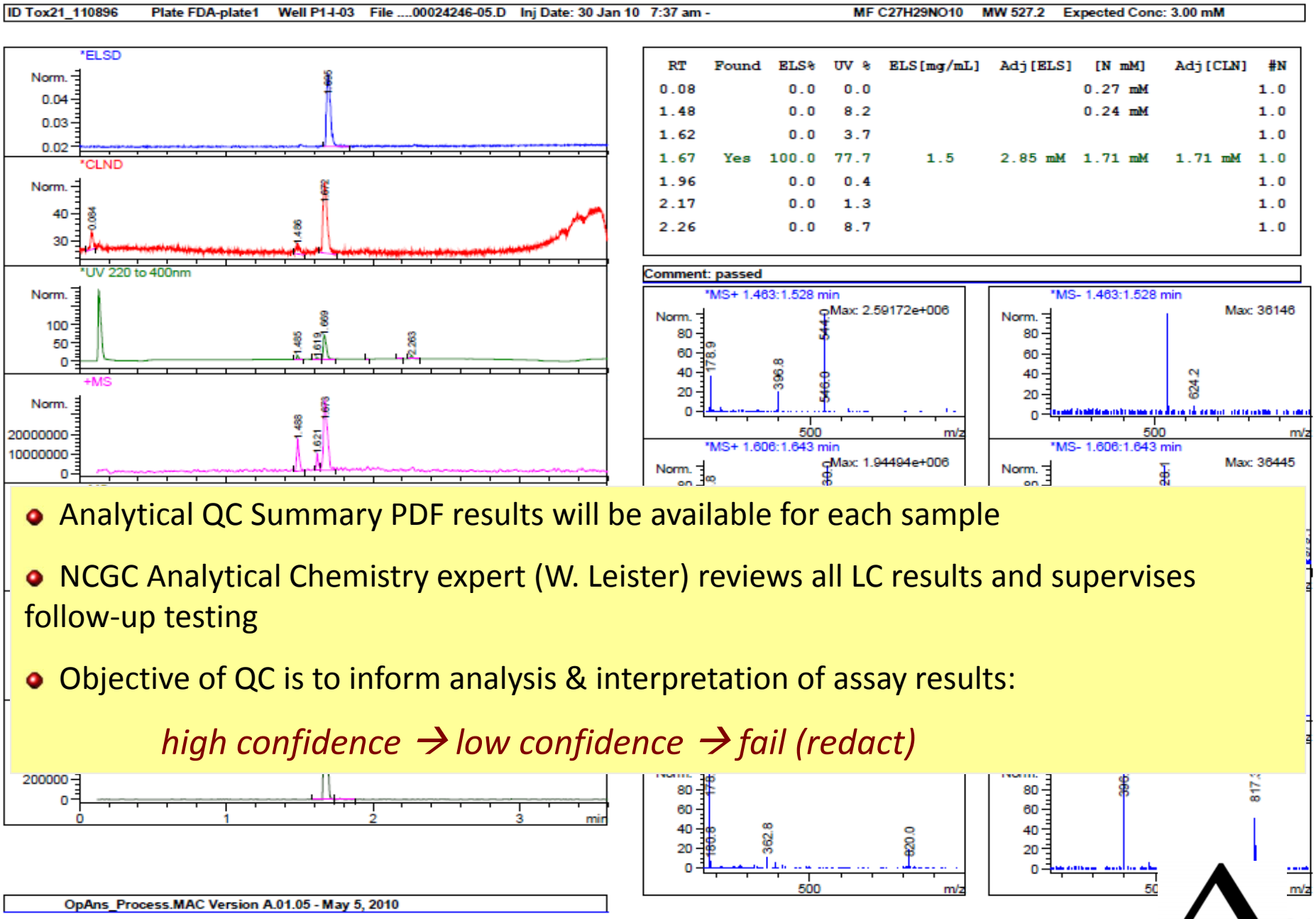
- Positive controls
- Time course
- Signal to background

Miniaturization

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

Attene-Ramos et al., 2013, Drug Discovery Today 18:716-723

- CV (coefficient of variation) = standard deviation (SD) of compound area/median of compound area
- Z factor = 1-[3*(SD of compound area + SD of basal)/(median of compound area – median of basal)]



- Analytical QC Summary PDF results will be available for each sample
- NCGC Analytical Chemistry expert (W. Leister) reviews all LC results and supervises follow-up testing
- Objective of QC is to inform analysis & interpretation of assay results:

high confidence → low confidence → fail (redact)

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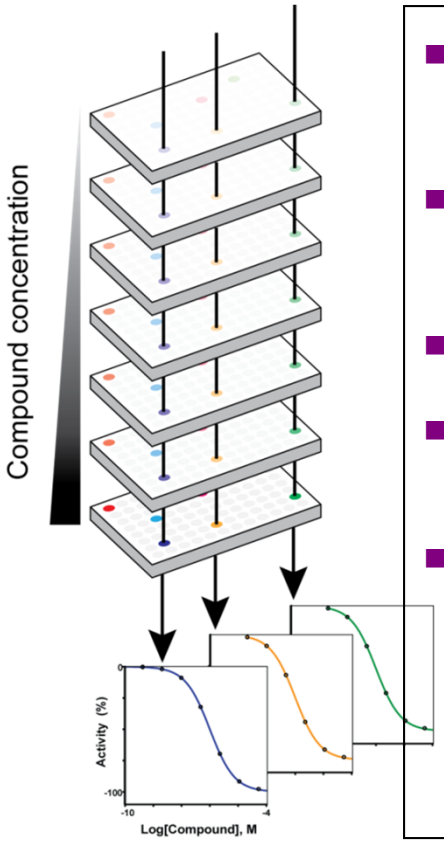
Tox21

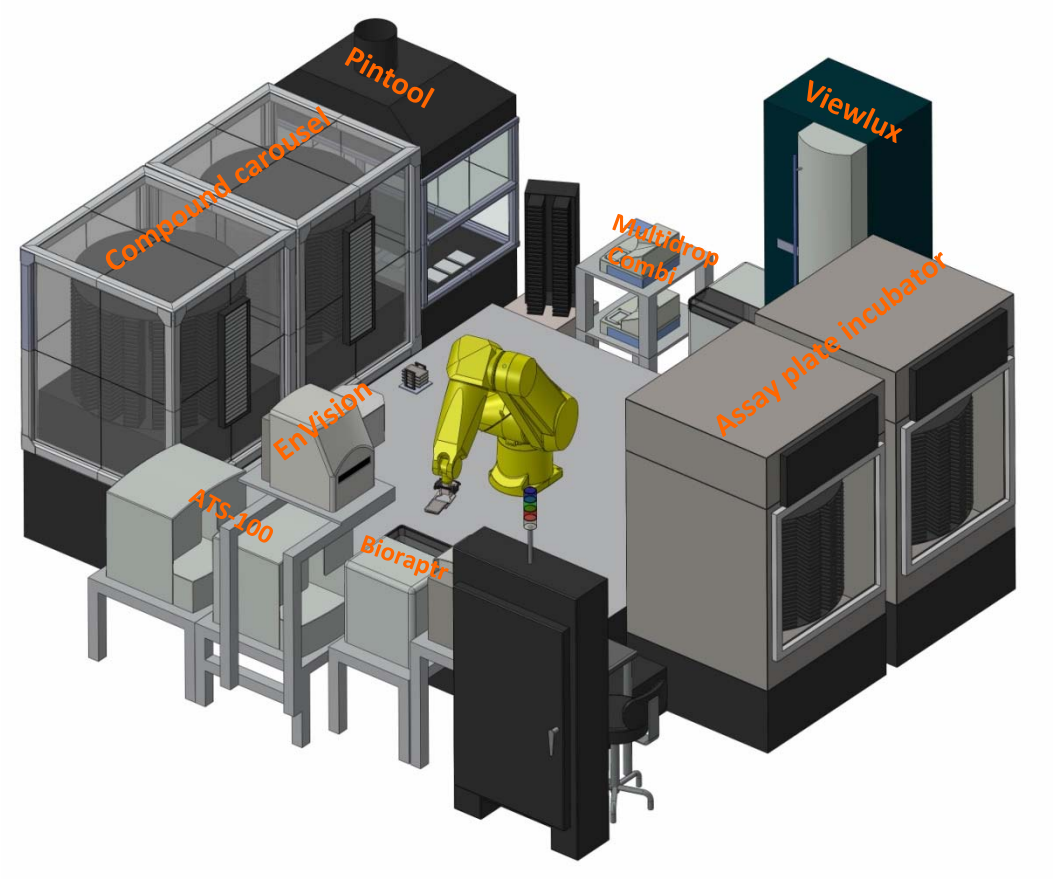
Quantitative High-Throughput Screening (qHTS)

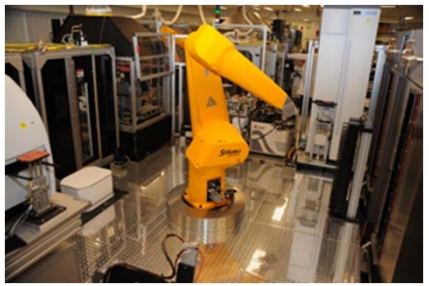


- Conventional screening done at one concentration
  - Not appropriate for toxicity testing – "dose makes the poison"
- qHTS tests compounds assayed at multiple concentrations
  - For Tox21, 14 concentrations over 4 logs (high:~ 100 uM)
- Miniaturized assay volumes 2-8 uL in 1536-well plate
- Informatics 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





ViewLux Multilabel Reader

- Absorbance
- Fluorescence
- F.P.
- Luminescence
- TR-FRET
- Top reading

EnVision Multilabel Reader

- Absorbance
- Fluorescence
- F.P.
- Luminescence
- TR-FRET
- AlphaScreen
- Top/Bottom reading

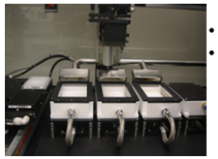
BioRAPTR FRD Workstation

- Transfer size: 0.2 - 10 ul
- 0.5 ml dead volume
- 4 reagents

Multidrop Combi

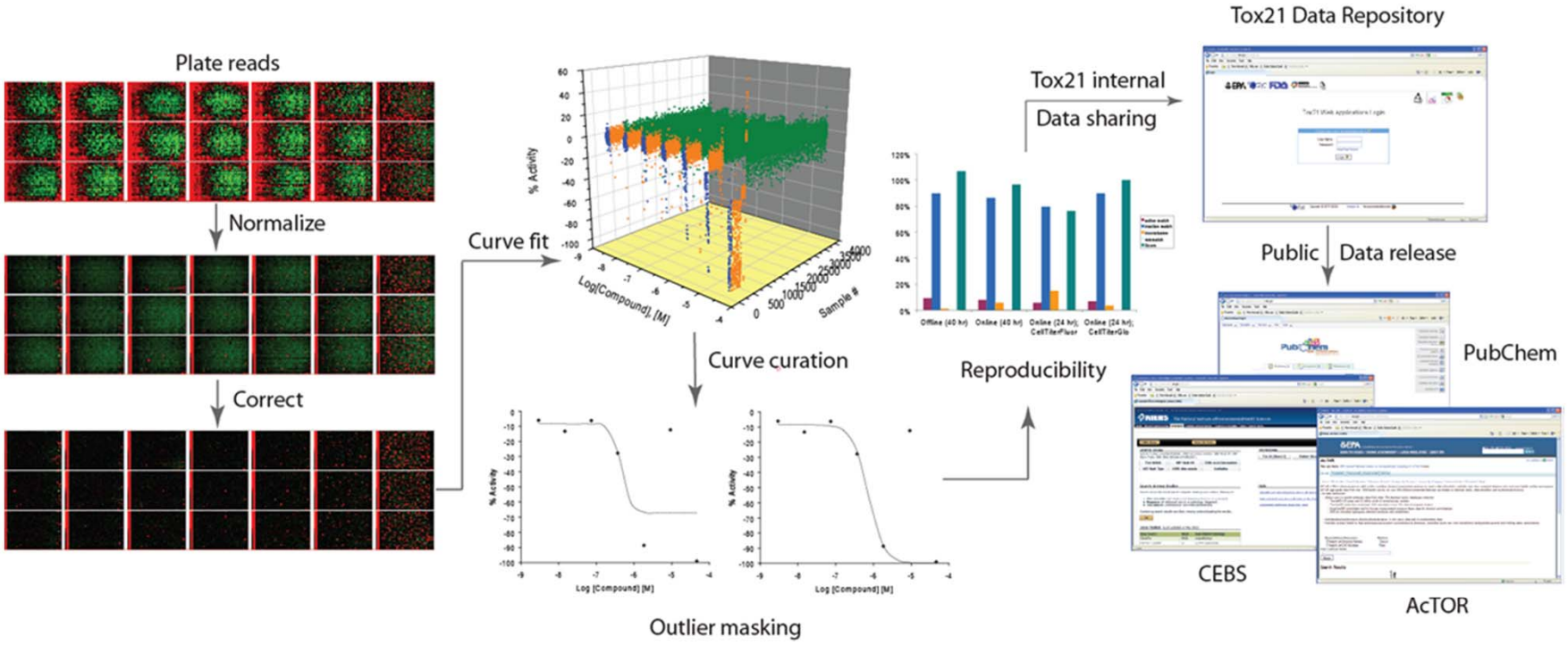
- Transfer size: 2 - 10 ul
- 10 ml dead volume
- 1 reagent

Pintool Station



- Transfe
- Pins washed in 3 solvents

Tox21 Informatics Analysis Process



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Tox21 Phase I
Proof of Principle, 2005-2008

- EPA via ToxCast™ screened 320 compounds (309 unique, primarily pesticide actives and some endocrine active compounds) in ~550 assays.

- NCGC screened 1408 compounds (1353 unique) from NTP and 1462 compounds (1384 unique) from EPA in >100 qHTS, largely cell-based reporter gene assays.

- Data released to the scientific community via:
  - EPA ACToR (Aggregated Computational Toxicology Resource; http://epa.gov/actor)
  - NLM PubChem (http://pubchem.ncbi.nlm.nih.gov/)
  - NTP CEBS (Chemical Effects in Biological Systems; http://www.niehs.nih.gov/research/resources/databases/cebs/index.cfm)

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Tox21 Phase II
Scale-Up, 2009-present

- EPA's ToxCast™ Phase II: ~700 compounds in >700 assays, ~1000 compounds in endocrine activity assays
- NCGC qHTS Phase II:
  - A 10K compound library screened 3 times at 15 concentrations in each assay
  - qHTS assays focused on:
    - nuclear receptor activation or inhibition
    - induction of cellular stress response pathways
- Characterizing human variability in *in vitro* responses

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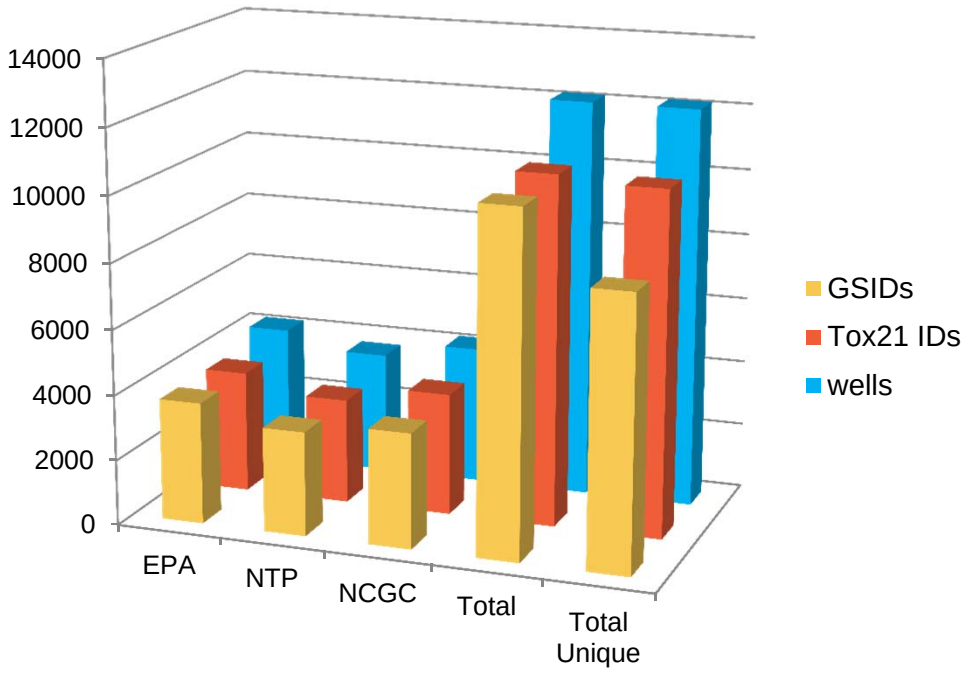
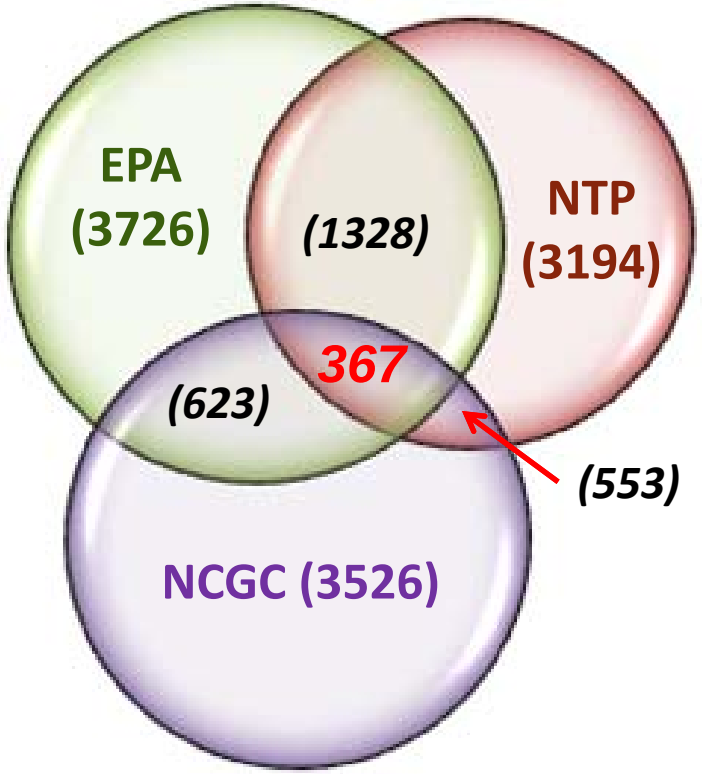
Tox21 10K Compound Library





Unique	EPA	NTP	NCGC	Total	Total Unique
GSIDs	3726	3194	3524	10444	8307
Tox21 IDs	3729	3210	3733	10672	10496
wells	4224	3726	4224	12174	12174

unique substances

unique solution IDs

total number of test cmpd wells

88 single-sourced cmpds in duplicate on each plate

2255 replicate substances (GSIDs) across 3 inventories

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Compound identity and structures available at http://www.epa.gov/ncct/dsstox/sdf_tox21s.html

Tox21 Phase II Limitations

- Extent of pathway coverage
- Focus on the use of reporter gene assays using immortal cell lines
- Extent of chemical coverage
- Focus on single compounds
- Limited capability for xenobiotic metabolism
- Focus on simple biological systems
- Limited to acute exposure scenarios
- Limited availability of high quality human toxicological data

Tox21 PubChem Data Deposition

- 66 Assay IDs
- 33 million data points of Phase II data have been deposited into PubChem

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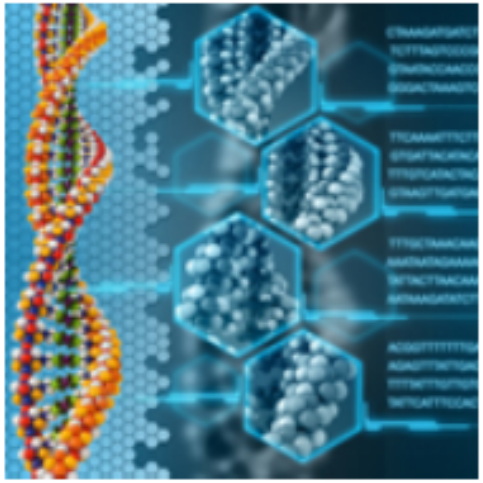
Tox21: *Release of Data on 1,800 Chemicals*



EPA Releases Chemical Screening Data on 1,800 Chemicals/Agency Improves Access to Chemical Data and Announces ToxCast Data Challenges

On Dec. 17, 2013, the U.S. Environmental Protection Agency (EPA) announced the release of toxicity data available through an interactive Chemical Safety for Sustainability (iCSS) Dashboard.

The tool provides user-friendly access to new information about 1,800 chemicals tested by NCATS researchers, as part of an ongoing federal collaboration called Toxicology in the 21st Century, or Tox21.

Tox21

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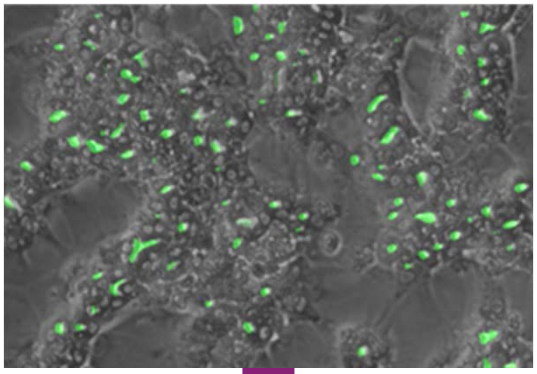
Tox21 Phase III

- Focus on high content 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
- Expanded utilization of lower organisms (zebrafish, C. elegans)

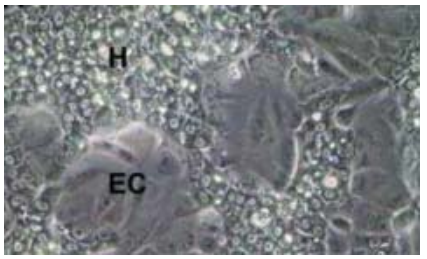


Targeted Assays

- 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

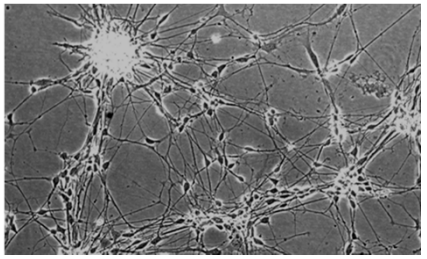
Tox21
Human Cell Lines for Toxicity Assessment

Hepatocytes:
- HepaRG,
- THLE-3 immortalized hepatocytes,
- Adult Liver Stem Cells(?)



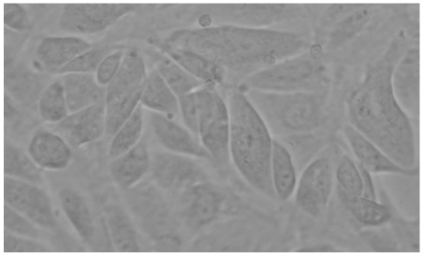
HepaRG

Neurons:
- SH-SY5Y,
- LUHMES conditionally immortalized dopaminergic neurons,
- Stem cell-derived neurons


iPS-derived neurons

Renal Proximal Tubule Cells:
- Tert-immortalized RPTEC

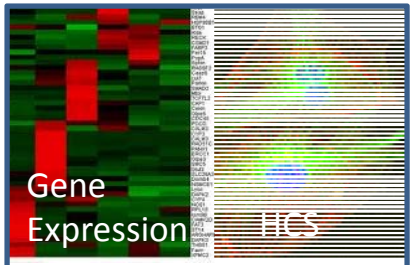
Listed cell lines have been cultured, evaluated using marker genes, and are in use to assess cytotoxicity

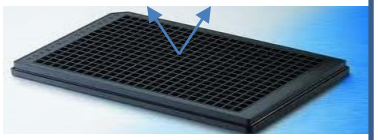

RPTEC/hTert

Tox21 Focused on Secondary Screening Needed to Bridge HTS to in vivo Toxicology

qHTS

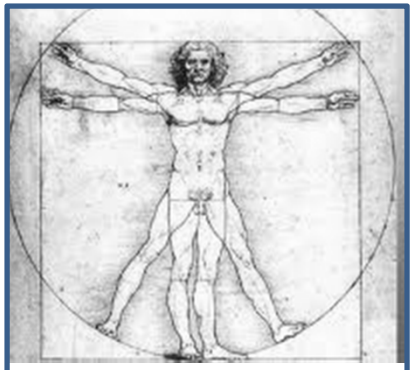
~10,000 cpds





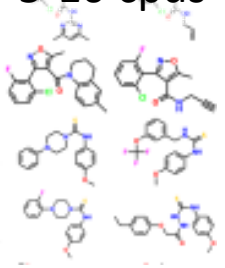
In vitro secondary assays

~100 cpds



Test in rats to predict human toxicity

5-10 cpds



Advancing Regulatory Science
NIH-FDA MOU 2010

- **Goal**
  - Accelerate development and use of new tools, standards and approaches to efficiently develop products and to more effectively evaluate product safety, efficacy and quality

- **Nature of Partnership**
  - Funded $6.75 million total costs for FY2010-2012, of which $750K from FDA and $6M from NIH
  - Expertise from both agencies helped shape the program and awards to reach goal

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Advancing Regulatory Science
Projects

- Accelerating Drug and Device Evaluation through Innovative Clinical Trial Design
- Development of a Replacement Ocular Battery (ROBATT)
- Characterization and Bioinformatics-modeling of Nanoparticle-Complement Interactions
- Development of a Heart-Lung Micromachine for Safety and Efficacy Testing

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Microphysiological Systems (MPS) Program
(aka, Tissue Chip, Organs-on-Chips)

- 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

NIH National Institutes of Health
Turning Discovery Into Health

DARPA

FDA

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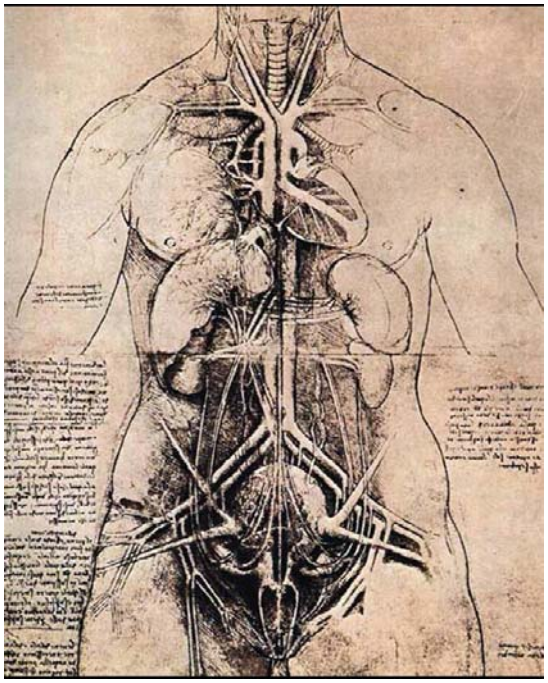
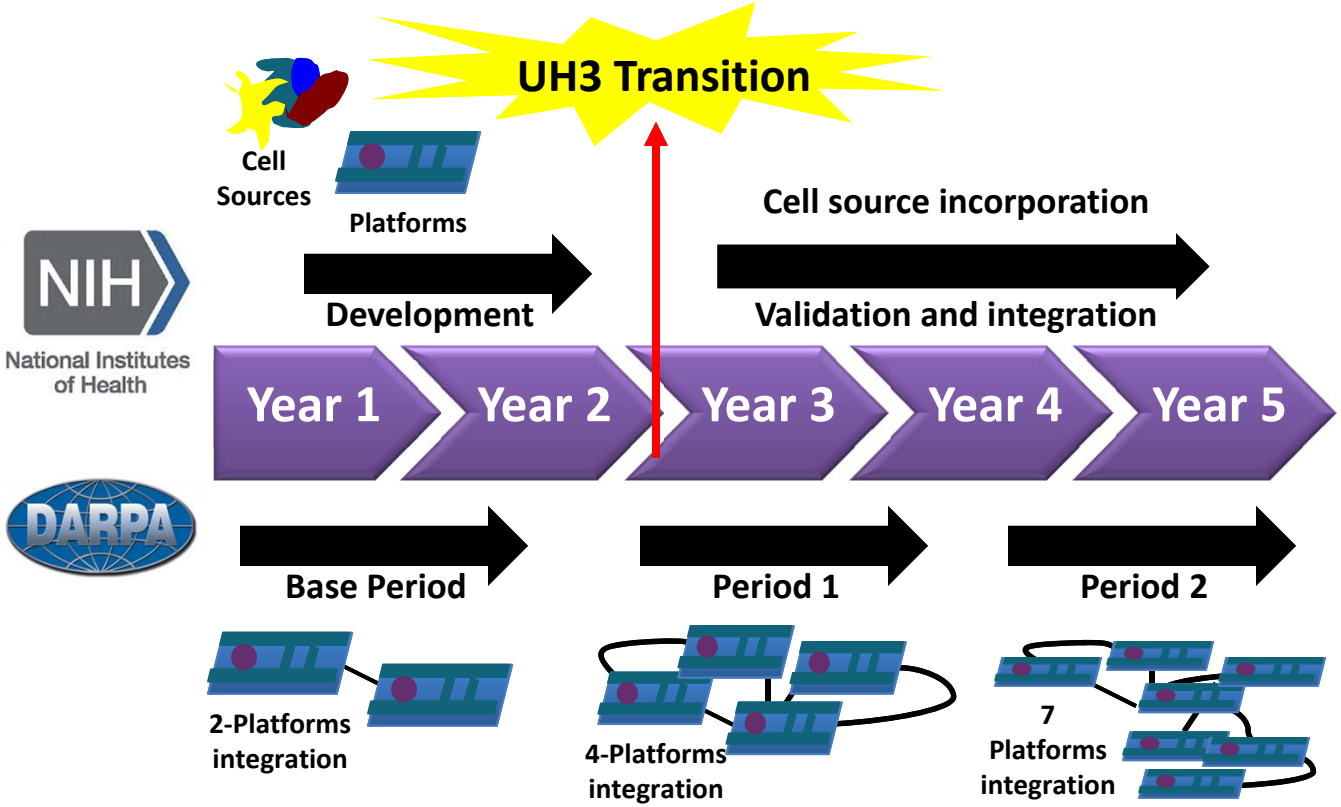
Microphysiological Systems Program

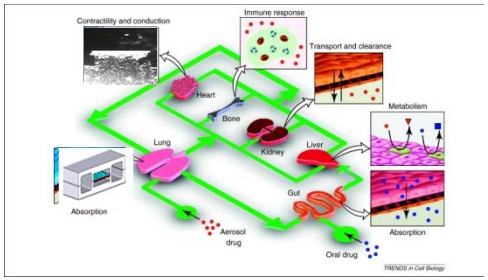
GOAL: Develop an *in vitro* platform that uses human tissues to evaluate the efficacy, safety and toxicity of promising therapies.



- 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.

MPS: Program Overview





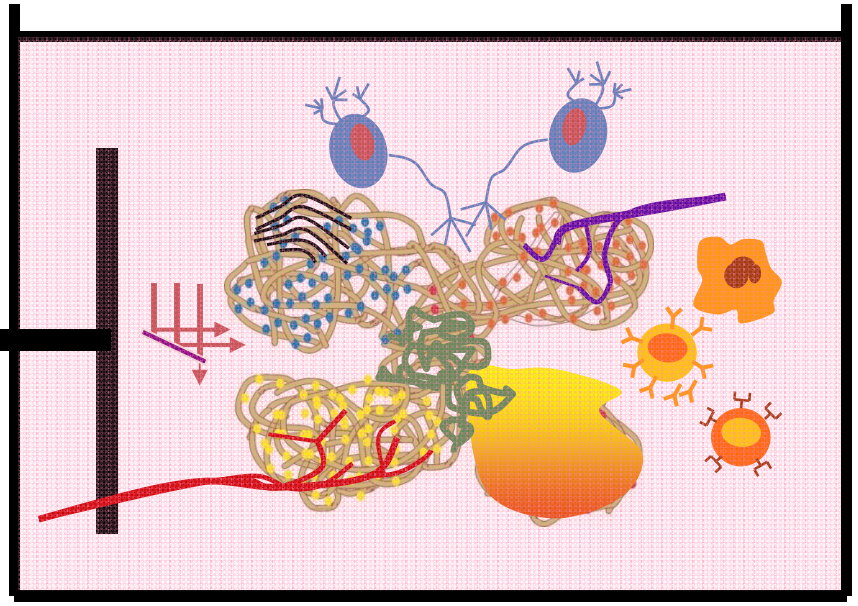
Body on a Chip

- Multi-cellular
- Vascularization
- Innervation
- Signaling

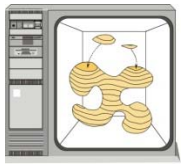
- Immune response
- Recapitulate normal and disease HUMAN PHENOTYPES

- 4 week cell viability
- Predicts human organ function and response
- Training compounds to validate systems

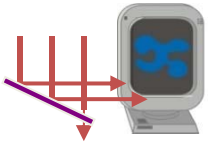
Microphysiological Systems from Common Building Blocks



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

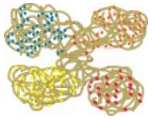


Functional Readout
- real-time, label-free, non-destructive sensing
- imaging

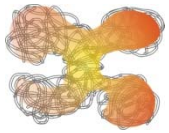


Scaffold
- purified ECM
- synthetic polymers
- composites

Cells
- stem/progenitor
- differentiated
- mixed cell types

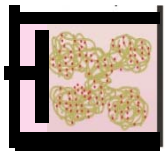


Structure
- porosity
- topography
- stiffness

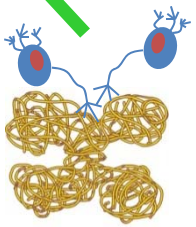


Spatial/Temporal Patterning
- cytokine gradients
- controlled release

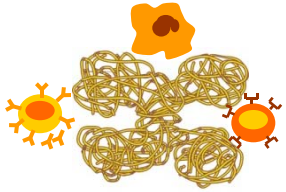
Perfusion
- embedded channels
- vascularization



Bioreactors
- optimized culture conditions
- biomechanical properties
- blood mimetics



Innervation
- signal propagation
- coordinated response



Host Response
- generalized inflammation
- specific immunity

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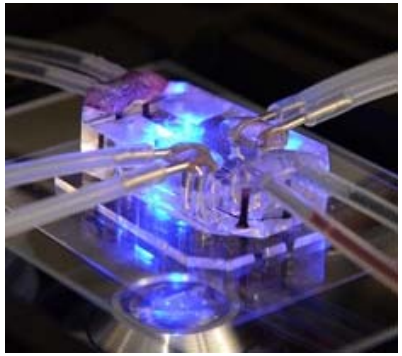
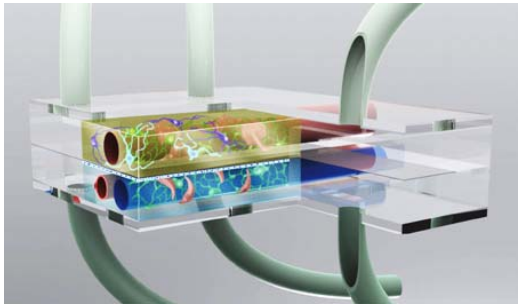
MPS Program

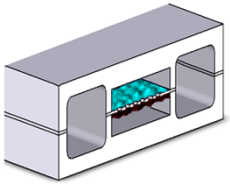
- Nineteen research project awards
  - Twelve to develop 3-D cellular microsystems representing human organ systems that will…
    - Be functionally relevant
    - Accurately reflect complexity of tissue of origin
  - Seven to explore potential of stem, progenitor cells to differentiate into multiple cell types
    - Representing cellular architecture within organ systems
    - Cells could populate tissue chips
- Program overview published in *Stem Cell Research and Therapy* December 20, 2013
  - Introduction authored by Margaret Sutherland, NINDS; and Dan Tagle & Kristin Fabre, NCATS
  - 18 review articles authored by awardees
  - Freely available at: http://stemcellres.com/supplements/4/S1



Lung chip
Wyss Institute

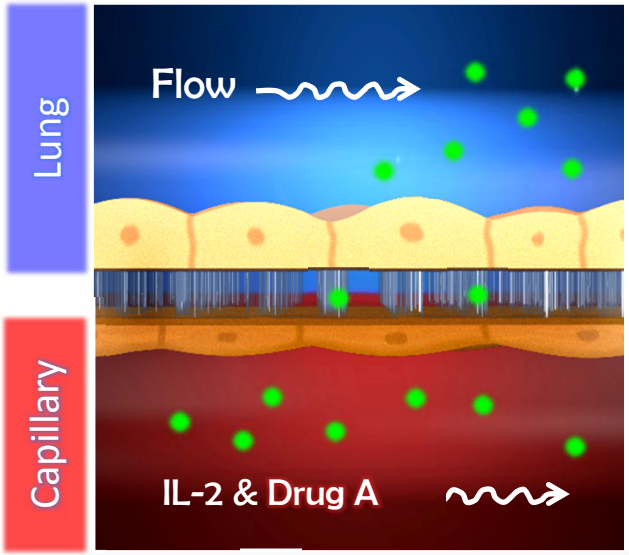


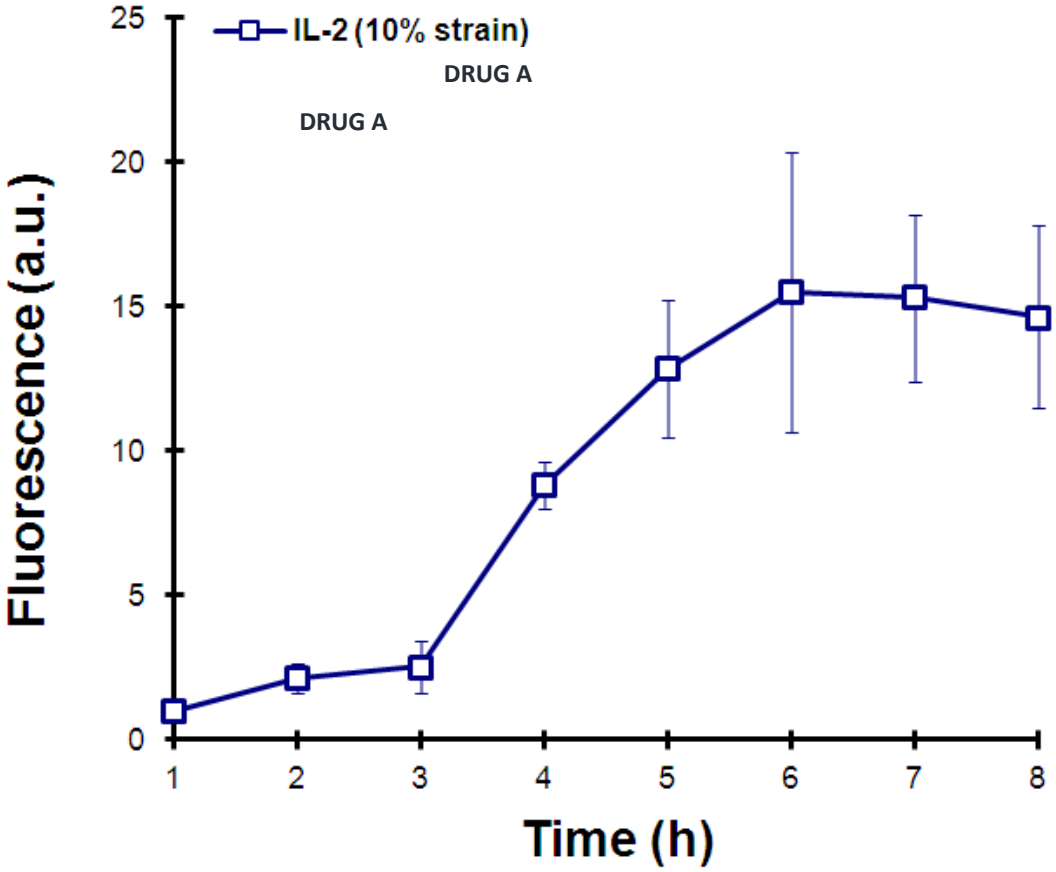
Blood-brain barrier chip
J. Wikswo, Vanderbilt



Lung-on-a-Chip: Predicting Drug Efficacy

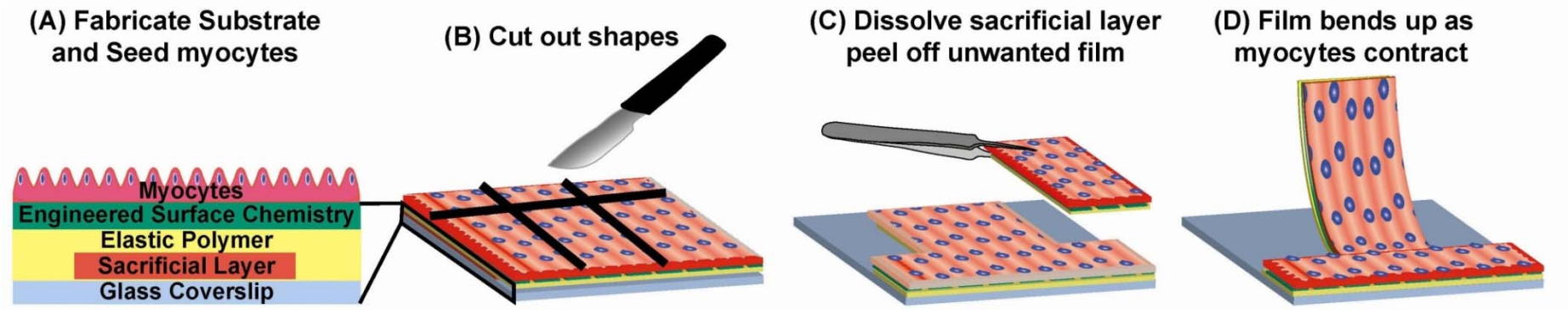
- Common side effect of IL-2 chemotherapy is vascular leak syndrome or edema

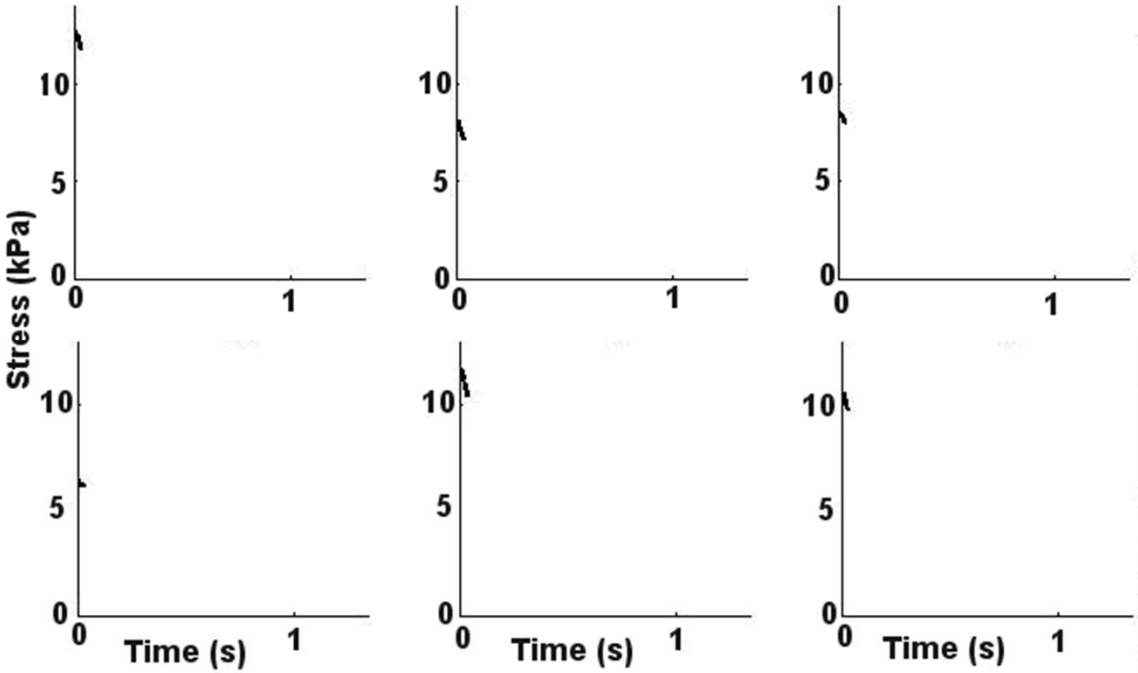


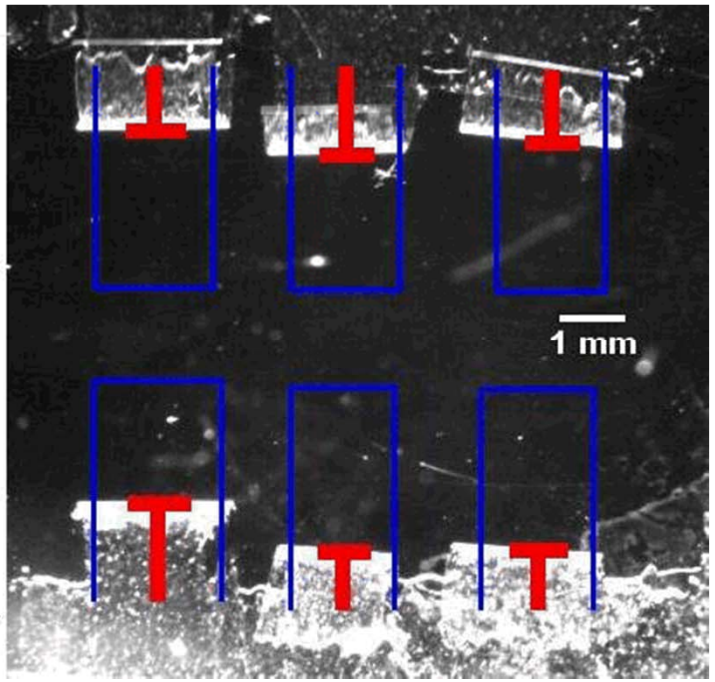


Data provided by Dr. Don Ingber, Wyss Institute

Engineered Cardiac Muscular Thin Films







Film length
Automatic projection tracking

Data provided by Dr. Kit Parker, Wyss Institute

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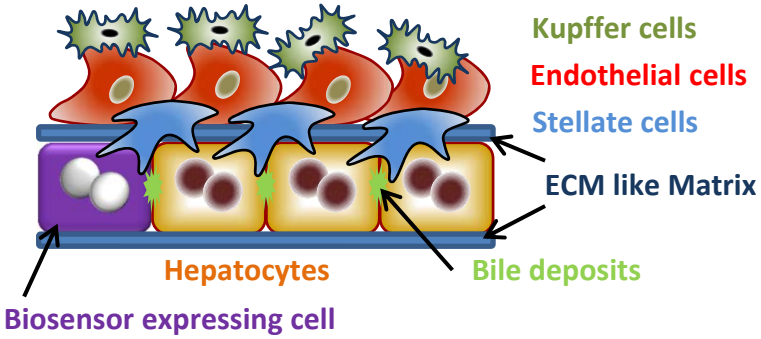
Science 2007;317:1366
Lab Chip 2011;11:4165

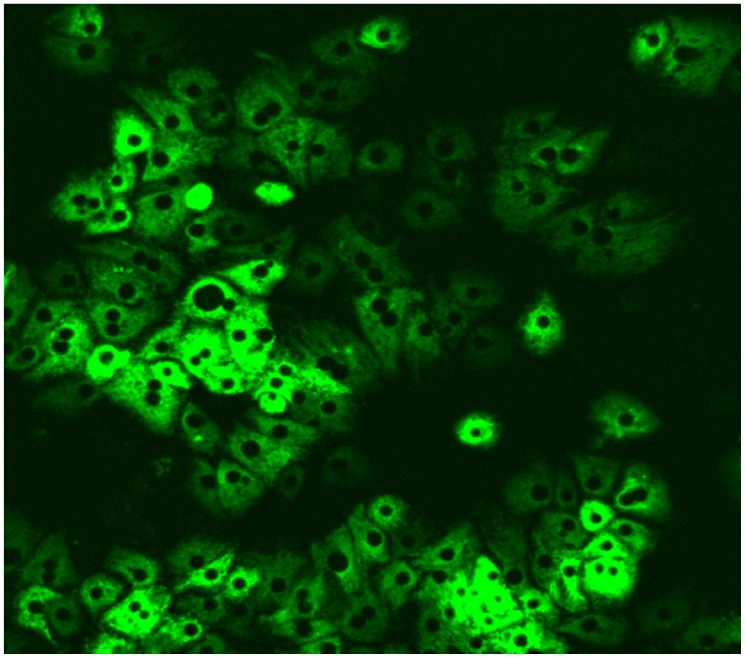
Biomaterials 2010;31:3613
J Pharm Tox Methods 2012;65:126

3-D biomimetic liver sinusoid construct



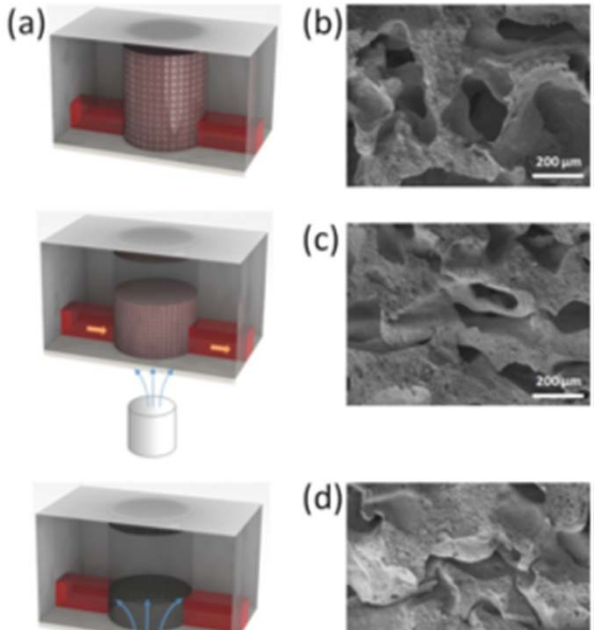
Sentinel cells: a subpopulation of hepatocytes, stellate and Kupffer cells that stably express biosensors to monitor key cell functions.

Biosensors

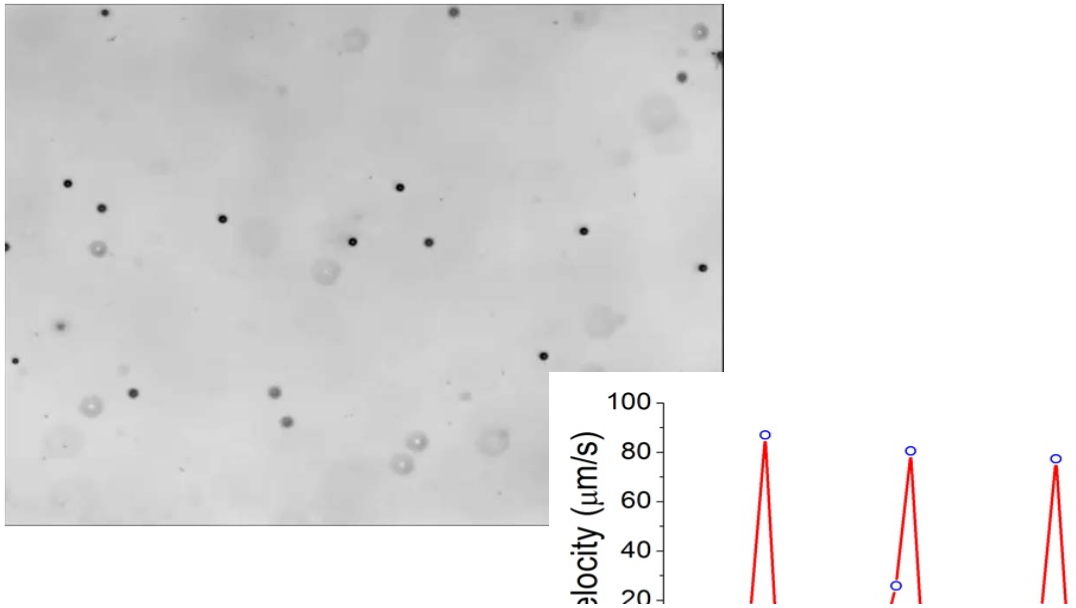


- **Cytochrome C released from mitochondria**
- **Exposed to 10µM Nefazadone**
- **Time-lapse of 16 hours**

Muscle and vasculature

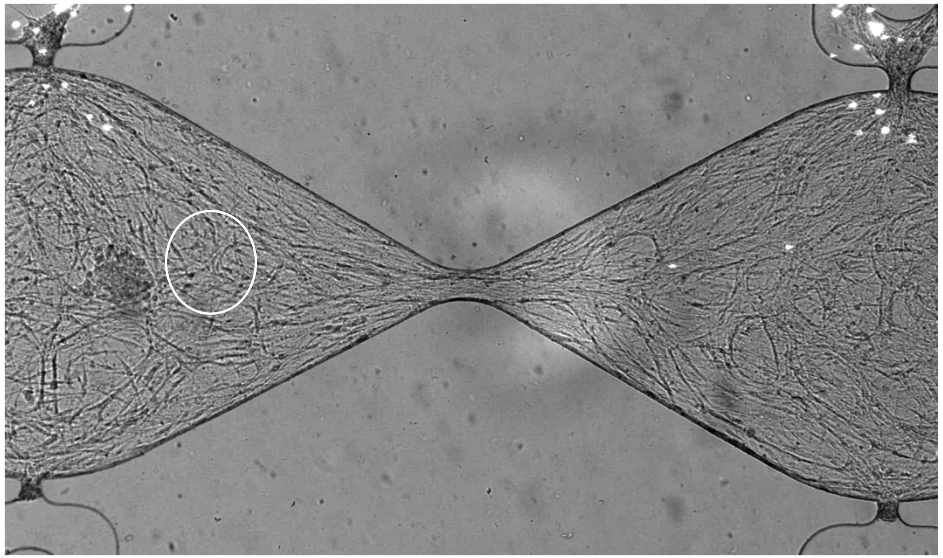


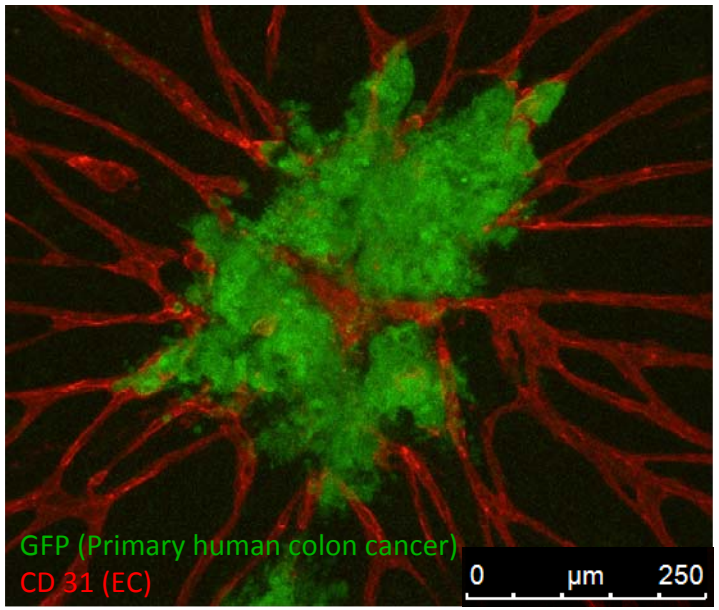
Recapitulating microvessel pumping



Perfused Tumor and Cardiac tissue

Perfused iPS-derived Capillaries with Tumor





- **7 days**
- **SW620 tumor spheroids**
- **hiPS-EC**
- **1 µm beads**

Cell Source Characterization

- **Up to 20 stem cell lines (ES and iPS) acquired, characterized, stored and distributed to MPS Project Members…including protocols**
- **Supported by NIH Common Fund; managed by NINDS and NCATS; collaboration with Coriell Institute**
- **Standardized characterization includes:**
  - sterility, including mycoplasma testing
  - normal growth and morphology characterization includes documentation of doubling time, freeze/thaw handling, and morphology.
  - Karyotyping
  - Surface antigen expression of stem cell markers (immunohistochemistry)
  - Embryoid body formation and TaqMan hPSC Scorecard analysis

CORIELL INSTITUTE
FOR MEDICAL RESEARCH

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NCATS

Training Compounds for Organs on Chips

- 100+ compounds with known toxic and/or biologic responses…compiled from MPS project members
- Compounds acquired, stored and distributed to MPS Project Members through Evotec
- Supported by NIH Common Fund; managed by NCATS; collaboration with Evotec..no cost to the MPS researchers

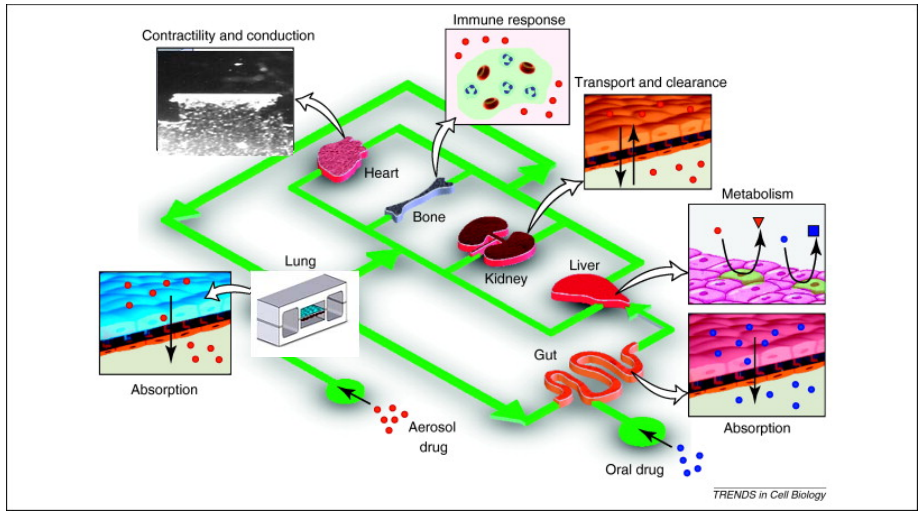
evotec

Drugs Producing Toxicity

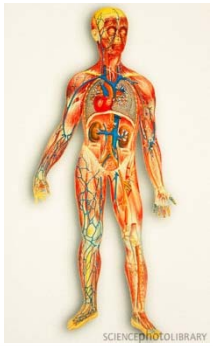
- Examples: Organ-specific toxicities:
  - Liver: hepatocyte injury, functional deficits (including metabolic changes, failure)
  - Heart: contractile failure, remodeling, lesions
  - Kidney: diuresis, proximal/distal tubular necrosis, failure
  - Pulmonary: obstruction, necrosis, edema

- System-dependent organ toxicities
  - Adverse systemic effects downstream from tissue with the drug receptor
  - Drugs for which metabolism is important
  - Drugs that induce a cytokine storm

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

DARPA and FDA Partnerships with NIH

DARPA

Barry Pallotta

Rebekah Cecil Gina Kost Amanda Lima

David Stepp

FDA

Kimberly Armstrong Thomas Colatsky Peter Goering Debra Lewis

Paul Brown Suzanne Fitzpatrick Paul Howard Brenton McCright

Melanie Blank Kevin Gaido Jean Hu-Primmer Tracy MacGill

Jeffrey Bray Abigail Jacobs Michael Santillo

NIH MPS Project Team

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Project Support – Kristin Fabre (NCATS)

Science Officers from NIH Institutes and Centers

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Guillermo Arreaza-Rubin (NIDDK)
David Balshaw (NIEHS)
Lisa Begg (ORWH)
Jill Carrington (NIDDK)
Michael Flessner (NIDDK)
Nancy Freeman (NIDCD)
Holli Hamilton (NIDCR)
Rosemarie Hunziker (NIBIB)
Jennie Larkin (NHLBI)
Sara Lin (NHLBI)
Nadya Lumelsky (NIDCR)
Martha Lundberg (NHLBI)
Matthew McMahon (NEI)
Melody Mills (NIAID)
Richard Okita (NIGMS)
Thomas Palker (NIAID)
Aaron Pawlyk (NIDDK)
Tom Peterson (CSR)
Douglas Sheeley (NIGMS)
Robert Star (NIDDK)
James Tricoli (NCI)
Hung Tseng (NIAMS)
Lois Tully (NINR)
Fei Wang (NIAMS)
Anne Zajicek (NICHD)

Medical Device Initiatives
NCATS Office of Rare Diseases Research

- **Trans-NIH Medical Device Research Interest Group**
  - NCATS/ORDR, NICHD, NIBIB, NHLBI, NIDDK, NIAMS, NIA, NEI, NINR, NINDS, NCI, FDA
  - Brookings Institution Medical Devices Forum Planning Group: Forum March 5th, 2014

- **Needs Assessment Project of Medical Devices for Rare Diseases**
  - Collaboration between ORDR and FDA Office of Orphan Products Development
  - Workshop January 8, 2014: "Complex Issues in Developing Medical Devices for Pediatric Patients Affected by Rare Diseases"
  - Planning and discussions continue

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Brookings Medical Device Innovation Forum
March 5th, 2014

Identifying challenges and prioritizing needs in medical devices research and development

- Participants addressed the challenges the medical device industry is facing in developing novel technologies that meet the needs of patients
- Topics covered
  - » Medical Device Innovation Grand Challenges
  - » Research and Pre-Clinical Development Challenges in the Current Environment Clinical Development, Product Approval, and Reimbursement
  - » Clinical Development, Product Approval, and Reimbursement
  - » Identifying Unmet Needs and Prioritizing Device Research

Planning Group: Brookings, NICHD, NCATS/ORDR

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Program Leads at NCATS

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