Health Effects Innovation at the National Toxicology Program

Brian R. Berridge, DVM, PhD, DACVP
Associate Director, NTP
Scientific Director, DNTP
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors Meeting
June 18, 2019
Aims of Health Effects Innovation

• Define and build a strategic assessment pipeline for key health effects

• Understand the mechanism of action, mode of action (MOA), health effect continuum for these areas

• Increase confidence in the predictivity of MOA assessments

• Align our capability development to problems we’re trying to solve

• Maximize the collective strength of the NTP organization

• Build novel partnerships in and outside NIH
Opportunity for Flipping the Paradigm

X lbs./yr. commercial production

Initial Focus

https://ncats.nih.gov/tissuechip/chip
Our Framework: Translational Toxicology Pipeline

- Hypothesis-driven Research
  - Data / Knowledge Mining
  - Computational Toxicology
  - Bioactivity Screening
  - In vitro Studies
- Knowledge Integration
- Chronic in vivo Studies
- Short term in vivo Studies
- Communicate
- Human Health Effects
Expected Outcomes (Benefactors)

- Novel capabilities (DNTP, Environmental toxicology community)
- Advancement of our current knowledge of how environmental exposures contribute to diseases of concern (Public health community)
- A paradigm shift (Environmental toxicology community)
- Peer-reviewed manuscripts (DNTP, individual contributing scientist)
- Presentations (DNTP, individual contributing scientist)
- Expertise (DNTP, individual contributing scientist)
Health Effects Innovation:
Cardiovascular Hazard Assessment in Environmental Toxicology

Brian R. Berridge, DVM, PhD, DACVP
Associate Director, NTP
Scientific Director, DNTP
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors Meeting
June 18, 2019
CV HEI Team

Scott Auerbach  
Biomolecular Screening Branch

Michelle Cora  
Cellular & Molecular Pathology Branch

Mark Cesta  
Cellular & Molecular Pathology Branch

Nicole Kleinstreuer  
NICEATM

Janine Santos  
NTP Laboratory

Kick-off Meeting on 29 May 2019

Brian Berridge  
DNTP Scientific Director
Cardiovascular disease is a major cause of death and disability worldwide. In the United States, heart disease and stroke are leading causes of death in both men and women and contribute significantly to direct health expenditures. The goal of the Cardiovascular Hazard Assessment in Environmental Toxicology HEI is to create a program that fully leverages and applies NTP’s capabilities in deliberate, integrated, and complementary ways along the toxicology translational pipeline—from data/knowledge mining to in silico/in vitro approaches to in vivo testing—and use that knowledge to improve our understanding about how environmental agents affect the cardiovascular system in humans.

Abstract- NTP BSC, June 2019
CV Health Effects Opportunities

Map cardiovascular ‘failure modes’ and agents

Mine current databases for CV-relevant MOA data

Define primary mechanistic screening strategy

Define and test Tier II 3D cardiomyocyte and vascular modeling systems

Instrumented animal studies for CV function assessment

Digital vivarium technology

NHLBI-NIEHS partnership

Contributing projects Partners and opportunities

Regulatory and public health stakeholders

Identify translational biomarkers of chronic CV health effects

NCATS, IQ, NCTR partnerships

Data / Knowledge Mining

Computational Toxicology

Bioactivity Screening

In vitro Studies

Short term in vivo Studies

Chronic in vivo Studies

Knowledge Integration

CV Evidence-based Testing Paradigm

Communicate

HESI Cardiac Safety Technical Committee

NCATS, IQ, NCTR partnerships

FDA ORISE Fellows

Identify translational biomarkers of chronic CV health effects

NTP BSC Presentation- Dec. 2018
A Novel Framework for Human-relevant and Failure Mode-based Assessment of Cardiovascular Safety in Nonclinical Drug Development

B. R. Berridge, DVM, PhD, DACVP
Co-Chair, HESI Cardiac Safety Technical Committee
Associate Director, NTP
Scientific Director, NIEHS DNTP
29 Mar 2019
MOU aims to improve cardiovascular safety of pharmaceuticals

NTP is part of a new interagency research collaboration to foster more novel, human-relevant safety testing methods

BY CAROL KELLY

Seeking to improve the cardiovascular safety of pharmaceuticals, the National Toxicology Program (NTP) partnered with the nonprofit Health and Environmental Sciences Institute (HESI) and the Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) in a new memorandum of understanding (MOU).

Who are the partners?

Brief descriptions of the MOU partner organizations and liaisons follow.
Contemporary pharmaceutical cardiovascular safety assessment would benefit from an approach that is more efficient in cost and time, mechanistically informative and human relevant. Such an approach would enable earlier recognition of development-limiting liabilities, fewer false positives leading to premature development termination, more relevant biomarkers and decreased late-stage attrition. The HESI Cardiac Safety Technical Committee will work across its working groups and with other stakeholders to design, test and implement such an approach.
• There are a finite number of primary responses to CV toxicity—i.e. failure modes (Principles of Pathology)
• Behind those failure modes, there are a finite number of key cellular and or molecular ‘mechanistic’ events (modes of action) that initiate and drive their pathogenesis which are ‘screenable’ (Principles of Molecular Biology)
• The likelihood of a xenobiotic inducing a failure mode is a product of its potency for functionally perturbing a cellular event and the likely in vivo exposure in dose and time (Principles of Toxicology)
  – our confidence in a phenotypic outcome for a mechanistic activity relates to our experience with it—i.e. some activity at a mechanistic level can be directly related to a phenotypic outcome (e.g. 5HT2b agonism) (Principles of Human Behavior)
  – other activities will require phenotypic confirmatory testing (i.e. Tier 2) in more complex biological systems to build confidence in the phenotypic outcome (Principles of Translational Toxicology Pipeline)
• A relevant mechanistic testing strategy should enable clinical risk assessment, progression decisions and the development of clinical monitoring strategies (Mission of NTP)
Reason to believe: We know a lot about the CV system

- Plumbing, electro-mechanics and power!
- Adaptive but not regenerative
- Relatively conserved
Reason to believe: We know a lot about what controls it

- **β-adrenergic agonist**
  - non-selective for $\beta_1$, $\beta_2$
  - $\beta_1 = \uparrow$ cardiac inotropy, chronotropy
  - $\beta_2 = \text{vasodilation}$

- Heart rate (chronotropy) determined by rate of spontaneous SA nodal discharge
- Spontaneous SA nodal discharge determined by balance of autonomic control

- Sympathetic: norepinephrine $\uparrow$ discharge
- Parasympathetic: acetylcholine $\downarrow$ discharge

- Frank-Starling Law
- Natriuretic peptides
- Renin-angiotensin system
- NO, Endothelin

**Abbreviations**: NE, norepinephrine; Gs, G-stimulatory protein; PK-A, cAMP-dependent protein kinase; SR, sarcoplasmic reticulum
Reason to believe: We know how it responds to injury

Structural injuries
- cardiomyocyte injury
- vascular injury
- valvulopathy
- organellar injury

Functional changes
- Arrhythmia
- ∆ BP
- ∆ HR
- ∆ contractility

Changes in disease
- Ischemic events
- Coronary artery dz
- Heart failure
- Cerebrovascular events
- Hypertension
- Metabolic disease
Acute/Single Dose Safety Pharmacology Studies

**In vitro/Ex vivo**
- Purkinje fiber assay
- Patch clamp ion channel assays
- Rabbit wedge assay

**In vivo**
- Rodents
  - Instrumented rat
  - Heart rate
  - ECG (quantitative)
    - No QT
  - Blood pressure
  - Contractility- LVP, QA

**Non-Rodents**
- FTIH-enabling GLP CV Study
  - Instrumented dog, mini-pig or monkey
  - Heart rate
  - ECG (quantitative)
  - Blood pressure
  - Contractility- LVP, QA

**Functional assessments**
- Individual animal; continuous recordings for 24+ hrs; Latin square design; dose-response; low 'n'
Current Paradigms in Preclinical Drug Safety Assessment

In Vivo Repeat-Dose General Toxicity Studies

Rodents

Morphologic endpoints
- Organ weight
- Gross
- Microscopic
  - light microscopy
  - ultrastructure

Blood/urine biomarkers
- Serum chemistry
- Hematology
- Hemostasis
- Urinanalysis

Non-Rodents

Functional endpoints
- Heart rate
- ECG
  (qualitative)

* Repeat dose studies biased toward morphologic endpoints
Leveraging Experiences- Mechanistic Screening Isn’t New!

Are there other targets we should be adding to this primary screen?

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Table 1 | Recommended targets to provide an early assessment of the potential hazard of a compound or chemical series

<table>
<thead>
<tr>
<th>Targets (gene)</th>
<th>Hit rate*</th>
<th>Binding</th>
<th>Functional or enzymatic</th>
<th>Main organ or system</th>
<th>Effects</th>
<th>Antagonism or inhibition</th>
<th>Refs</th>
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<td>G-protein coupled receptors</td>
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<tr>
<td>Adenosine receptor A (ADORA1)</td>
<td>High</td>
<td>CV, CNS</td>
<td>Channel-mediated effects</td>
<td>Corneal edema, photophobia, miosis</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>57</td>
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<tr>
<td>a1-adrenergic receptor (ADR A2)</td>
<td>High</td>
<td>CV, CNS</td>
<td>vasoconstriction</td>
<td>vasoconstriction, negative chronotropic effect</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>55</td>
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<td>a2-adrenergic receptor (ADR A2)</td>
<td>High</td>
<td>CV, CNS</td>
<td>vasoconstriction</td>
<td>vasoconstriction, negative chronotropic effect</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
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<td>G-protein coupled receptors (cont.)</td>
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<tr>
<td>Muscarinic acetylcholine receptor M (CHRM)</td>
<td>High</td>
<td>Low (agonist); high (antagonist)</td>
<td>CNS, GI, CVS</td>
<td>Cognition/learning, memory, mood, sleep</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>73</td>
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<tr>
<td>Muscarinic acetylcholine receptor M (CHRM)</td>
<td>High</td>
<td>Low (agonist); medium (antagonist)</td>
<td>CVS</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>Tachycardia, bronchoconstriction, tachyphylaxis, tachyphylaxis, tachyphylaxis</td>
<td>74</td>
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<tr>
<td>Muscarinic acetylcholine receptor M (CHRM)</td>
<td>High</td>
<td>NA</td>
<td>GI, pulmonary</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>Constipation, blurred vision, pupillary dilation, dry mouth</td>
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<tr>
<td>5-HT1a (HT1 A2)</td>
<td>Medium/high</td>
<td>Low (agonist); medium (antagonist)</td>
<td>CNS, endocrine system</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>76</td>
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<tr>
<td>5-HT1a (HT1 A2)</td>
<td>High</td>
<td>High (agonist); medium (antagonist)</td>
<td>CNS, GI, CVS</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>Tachycardia, bradycardia, hypotension, tachycardia, bradycardia, hypotension, tachycardia, bradycardia, hypotension</td>
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<tr>
<td>5-HT1a (HT1 A2)</td>
<td>Very high</td>
<td>Low (agonist); medium (antagonist)</td>
<td>CNS, GI, pulmonary</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>78</td>
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<tr>
<td>5-HT1a (HT1 A2)</td>
<td>High</td>
<td>Very low</td>
<td>CNS, endocrine system</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>79</td>
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<tr>
<td>Vasopressin V1a receptor (VPA)</td>
<td>Medium</td>
<td>High</td>
<td>Renal, CVS</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>Potential for stimulation of platelet aggregation, inhibition of bronchial smooth muscle contraction</td>
<td>80</td>
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</tr>
</tbody>
</table>

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- **A Guide to Drug Discovery — Opinion**

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**Reducing safety-related drug attrition: the use of in vitro pharmacological profiling**

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</tr>
</thead>
<tbody>
<tr>
<td>Cannabinoid receptor CB1 (CB1)</td>
<td>Medium/high</td>
<td>Medium (agonist)</td>
<td>CNS</td>
<td>Epilepsy, mood disorders</td>
<td>tachyphylaxis, tachyphylaxis, tachyphylaxis, tachyphylaxis</td>
<td>63</td>
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<tr>
<td>Cannabinoid receptor CB2 (CB2)</td>
<td>Medium</td>
<td>Medium (agonist)</td>
<td>Immune</td>
<td>Inflammation, immune response</td>
<td>Inflammation, immune response</td>
<td>63</td>
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<tr>
<td>Cholecystokinin A (CCK-A)</td>
<td>Low/medium</td>
<td>NA</td>
<td>GI</td>
<td>Inhibition of gastric acid secretion, increase in GI motility</td>
<td>Tachyphylaxis, tachyphylaxis, tachyphylaxis, tachyphylaxis</td>
<td>64</td>
<td></td>
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<tr>
<td>Dopamine receptor D1 (D1D1)</td>
<td>Medium</td>
<td>High (agonist)</td>
<td>CNS, CVs, GI, Endothelin receptors A (EDNRA)</td>
<td>Vascular relaxation, inhibition of platelet aggregation</td>
<td>Tachyphylaxis, tachyphylaxis, tachyphylaxis, tachyphylaxis</td>
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<tr>
<td>Dopamine receptor D2 (D2D2)</td>
<td>Medium/High</td>
<td>High (agonist); medium (antagonist)</td>
<td>CVs, CNS, Endothelin receptors A (EDNRA)</td>
<td>Vascular relaxation, inhibition of platelet aggregation</td>
<td>Tachyphylaxis, tachyphylaxis, tachyphylaxis, tachyphylaxis</td>
<td>65</td>
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<tr>
<td>Endothelin receptors A (EDNRA)</td>
<td>Low</td>
<td>NA</td>
<td>CVs, development</td>
<td>Vascular relaxation, inhibition of platelet aggregation</td>
<td>Tachyphylaxis, tachyphylaxis, tachyphylaxis, tachyphylaxis</td>
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</tbody>
</table>
In vitro cardiotoxicity assessment of environmental chemicals using an organotypic human induced pluripotent stem cell-derived model

Oksana Sirekno, Fabian A. Grimm, Kristen R. Ryan, Yasuhiro Iwata, Weihua A. Chiu, Frederick Parham, Jessica A. Wignall, Blake Anson, Evan F. Cromwell, Manita Behl, Ivan Rusyn, Raymond R. Tice

Reason to Believe: Technology
**Reason to Believe: Technology**

**Advanced in vitro models of vascular biology: Human induced pluripotent stem cells and organ-on-chip technology**

Amy Cochrane, Hugo J. Albers, Robert Passier, Christine L. Mummery, Albert van den Berg, Valeria V. Orlova, Andries D. van der Meer

<table>
<thead>
<tr>
<th>Vascular Barrier Models</th>
<th>Hemostasis &amp; Thrombosis</th>
<th>Vasculogenesis &amp; Angiogenesis</th>
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**2D Vasculature**

- A
- B

**3D Vasculature**

- C
- D
- E
- F
- G
- H
- I
High Performance Computing Applications

Presented to: Secretary of Energy Advisory Board (SEAB)

Fred Streitz, Director
HPC Innovation Center
Lawrence Livermore National Laboratory

December 3, 2013

Whole heart modeling at cellular resolution in real time
Example of joint capability development

- Bring together multi-disciplinary, multi-institutional team (IBM-LLNL)
- Develop high resolution, realistic model of human heart
- Create Cardioid code to model electrophysiology of heart
- Leverage 20 PF Sequoia (Blue Gene/Q) resource
- Investigate development of arrhythmia

“Bake out” period on new architecture enables discovery-class open science
Publishable, newsworthy work returns favorable press for DOE and NNSA
Scaling, load balance and resilience issues are worked out prior to program use
Reason to Believe: Smart Scientists

• Arif Rahman, PhD
  – FDA ORISE Fellow
  – Mentor = Scott Auerbach
  – Project- Develop a WGCNA (Whole Genome Co-Expressed Network Analysis) tool for CV hazard assessment

• Shagun Krishna, PhD
  – DNTP Post-Doc Fellow
  – Mentor = Nicole Kleinstreuer
  – Project- Develop computational CV QSAR tools and integrated testing strategies using publicly-available data (e.g. Tox21)
Linking Mechanisms to Phenotypes

E.g. Calcium handling, contractility and heart failure
Linking Mechanisms to Phenotypes

E.g. Calcium handling, contractility and heart failure

Screen this

Model this

Predict this

WGCNA, In vitro, In vivo studies
• A visualization tool to understand chemical activity against the available *in vitro* assays in ToxCast HTS database with CV related targets.

• Rank chemicals based on bioactivity against these targets

• Available data in Toxcast (invitroDB_v3)
  - 9215 Chemicals (Tox21/ToxCast library, tested to varying degrees in the target assays)
  - 66 *in vitro* assays that map to pharmacological screening targets for 9 families of selected proteins
  - Generate CardioToxPi and provide chemical bioactivity ranking against selected targets
• CardioToxPi ranked 9215 chemicals based on ToxCast Score (negative log scaling):
  
  • ToxPiScore Range: 0.6260 - 0
    - 0.620 - 0.10 (120 Chemicals)
    - 0.10 - 0.00 (9095 Chemicals)
Work is ongoing to expand the ToxPi to include phenotypic endpoints such as Mitochondrial Function, Oxidative Stress, VEGF Pathway, and Vascular Cell Cytotoxicity

S. Krishna
DrugMatrix - Cardiac Gene Modules

- Node Group 1: Cellular stress/Inflammation
- Node Group 2: Metabolic/stress response/EV
- Node Group 3: Immune response
- Node Group 4: Extracellular matrix
- Node Group 5: Metabolism/Myocyte repair/hypertrophy
- Node Group 6: Cell cycle/division
- Node Group 7: Tissue morphogenesis
- Node Group 8: Protein/lipid synthesis
- Node Group 9: Energy/heat production/metabolism
- Node Group 10: Lipid metabolism
- Node Group 11: Iron homeostasis/muscle contraction
- Node Group 12: Circadian/cardiac rhythm
- Node Group 13: Carbohydrate metabolism
- Node Group 14: Lipid/protein metabolism
- Node Group 15: Cardiac remodeling/carcinogenesis
- Node Group 16: Inflammation
- Node Group 17: Immune regulation
- Node Group 18: Protein maturation
- Node Group 19: Heme biosynthesis

A. Rahman
Associating changes in gene modules of relevance to the CV system with phenotypic evidence of adaptation, injury and progression.

A. Rahman
Define discrete problem statements
Scope the biology/pathobiology of interest
Capability and knowledge gap analysis
  Map stakeholders
  Map current capabilities (internal and external) and data sources
  Identify what parts of a functional pipeline are missing
Identify contemporary environmental toxicology problems to which to align capability development
  E.g. PFAS, BPA, PAHs
Develop a CV HEI Program/Project Strategic Plan
  Presentation to DNTP LT Governance Committee
Build a project and data management framework
Questions?