Dept Environmental Health and Engineering,
Bloomberg School of Public Health – Johns Hopkins University
June 11, 2021 (webinar)

Researching Developmental Toxicity Models for Children's Environmental Health

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DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

Virtual Tissue Models

The VTM Research Area will provide physical models and mathematical simulations of specific organ systems and developmental outcomes informing risk-based assessments of new and existing chemicals. This research area expands understanding of chemical effects on developmental and reproductive toxicology.

Outputs

CSS 5.1 (C Deisenroth)
CSS 5.2 (S Hunter)
CSS 5.3 (T Knudsen)

CSS 5.3– Computational VTMs

Output Lead: knudsen.thomas@epa.gov
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Product Leads:
Tom Knudsen (CCTE-BCTD)
Imran Shah (CCTE-BCTD)
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Contractors:
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Richard Spencer (EMVL)
ArunA Biomedical
Stemina Biomarker Discovery
Vala Sciences

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Chad Deisenroth (CCTE- BCTD)
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Students / Fellows:
Todor Antonijevic (now ToxStrategies)
Bryant Chambers (R-postdoct, BCTD)
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Katerine Saili (now OAQPS)
Todd Zurlinden (now CEPHEA)

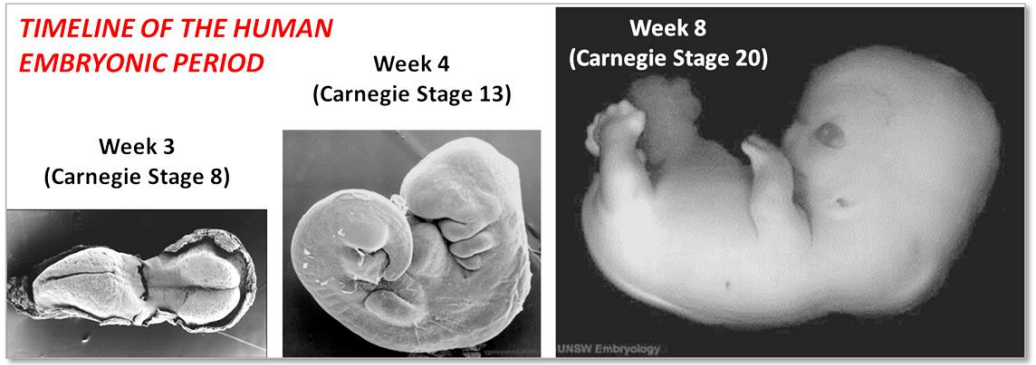
Program Partners:
Monique Perron (OPP)
Todd Stedeford (OPPT)
Sue Euling (OCHP)

Collaborators:
Jianping Fu (Univ Michigan)
James Glazier (Indiana Univ)
William Murphy (Univ Wisconsin)
Tox21 CPP #6 and CPP #13
OECD-WNT-TGP

Evaluating chemical effects on the developing embryo

"The first trimester is the most crucial to your baby's development. During this period, your baby's body structure and organ systems develop."

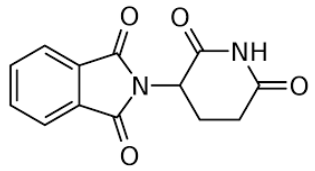
www.ucsfhealth.org



peak sensitivity (3rd – 8th wk)

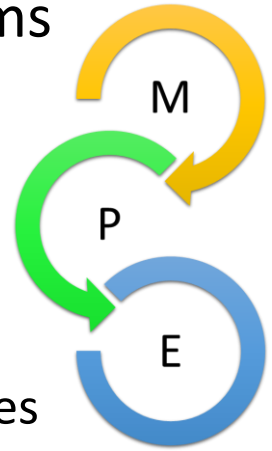
Embryonic Period	***Fetal Period***	***Fetal Period***
T1	**T2**	**T3**

OECD TG 414
OPPTS 870.3700



Adverse Birth Outcomes (CDC)
- preterm birth rate (10%)
- low birth weight babies (11%)
- malformations (3-4% live births)
- functional deficits (17% children)
- mortality (1-2%)

Complex Systems
- gene networks
- multiscale
- autopoiesis
- canalization
- temporality
- state trajectories
- and more …



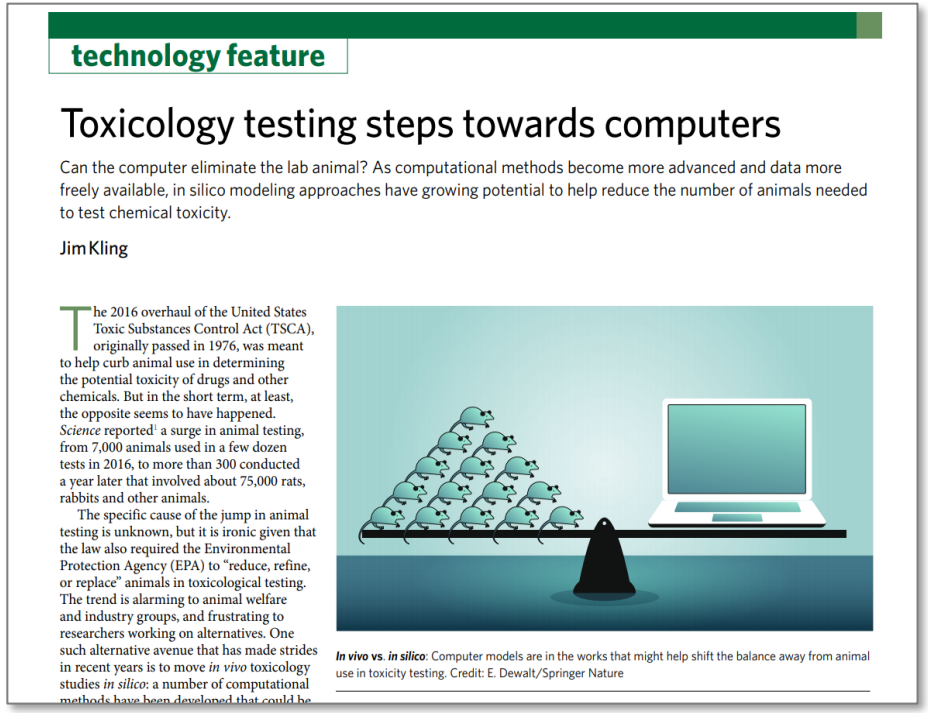
Shifting toxicity testing to animal-free alternatives

- **June 2016:** *Lautenberg Chemical Safety Act* advances chemical safety evaluation with methods that reduce animal testing and are translatable to vulnerable populations & lifestages.
- **September 2019:** directive issued by USEPA Administrator Wheeler set a vision to reduce mammalian study requests 30% by the year 2025 and eliminate them by 2035.
- **June 2020:** USEPA work plan to accelerate scientifically valid *New Approach Methods* (NAMs) for assessing toxicity of large numbers of chemicals with less reliance on animal testing.

https://www.epa.gov

In vitro data and *in silico* models that reflect key aspects of embryo-fetal development will be indispensable for NAM-based detection of developmental hazard potential.

Can the computer replace lab animal testing?



technology feature

Toxicology testing steps towards computers

Can the computer eliminate the lab animal? As computational methods become more advanced and data more freely available, in silico modeling approaches have growing potential to help reduce the number of animals needed to test chemical toxicity.

Jim Kling

The 2016 overhaul of the United States Toxic Substances Control Act (TSCA), originally passed in 1976, was meant to help curb animal use in determining the potential toxicity of drugs and other chemicals. But in the short term, at least, the opposite seems to have happened. *Science* reported[1] a surge in animal testing, from 7,000 animals used in a few dozen tests in 2016, to more than 300 conducted a year later that involved about 75,000 rats, rabbits and other animals.

The specific cause of the jump in animal testing is unknown, but it is ironic given that the law also required the Environmental Protection Agency (EPA) to "reduce, refine, or replace" animals in toxicological testing. The trend is alarming to animal welfare and industry groups, and frustrating to researchers working on alternatives. One such alternative avenue that has made strides in recent years is to move *in vivo* toxicology studies *in silico*: a number of computational methods have been developed that could be

In vivo* vs. *in silico: Computer models are in the works that might help shift the balance away from animal use in toxicity testing. Credit: E. Dewalt/Springer Nature

Nature (Lab Animal) 48: 40-42, February 2019

A 2019 technology feature in *Nature - Lab Animal* highlighted progress toward the animal-free zone.

- **Mapping the (chemical) world:** structural alerts based on 'black box' and expert read-across.
- **Opening the black box:** performance-based weight of evidence models from *in vitro* profiling.
- **A step further:** 'virtual embryo' computer models that simulate cellular changes on development.

Understanding strengths and limitations for predictive toxicology:

1) *in vitro* testing with human pluripotent stem cell (hPSC) models;
2) expanding the 'virtual embryo' toolbox for predictive toxicology.

1. Pluripotent stem cell (hPSC) models

An active area of investigation and one of the most promising *in vitro* alternatives to pregnant animal testing for assessing developmental hazard potential; novel features:

- **Self-renewal:** cells replicate themselves indefinitely when cultured under appropriate growth factor conditions.
- **Pluripotency:** cells have the potential to form most of the different cell types comprising the embryo/fetus.
- **Autopoiesis:** capacity to self-organize into rudimentary tissues and more complex organoid structures.

Established hPSC lines can recapitulate **some** biology driving embryogenesis during the period covered by guideline prenatal studies (e.g., OECD TG 414, OPPTS 870.3700).

A few milestones that set the stage for hPSC platforms …

1975: the term ‘ESC’ was first coined by research pioneers to distinguish pluripotent cells derived from an early mouse embryo versus pluripotent embryonal carcinoma cells.

1998: ESCs isolated from human blastocysts and cultured under conditions to maintain self-renewal still form derivatives of all 3 embryonic germ layers after 4-5 months.

2001: ethical debate led POTUS to issue an executive order (EO 13505) limiting federally-funded research on hESC lines to 21 cell lines established before August 9, 2001.

2006: discovery that dermal fibroblasts could be reprogrammed to a pluripotent state (iPSCs) simply by altering expression of 4 genes (Oct3/4, Sox2, c-Myc, Klf).

https://stemcelldb.nih.gov/ NIH database of genomic profiling data on the 21 hESC lines approved under GW Bush administration as well as registered human iPSC lines.

Disclosures

https://stemcells.nih.gov/research/registry.htm

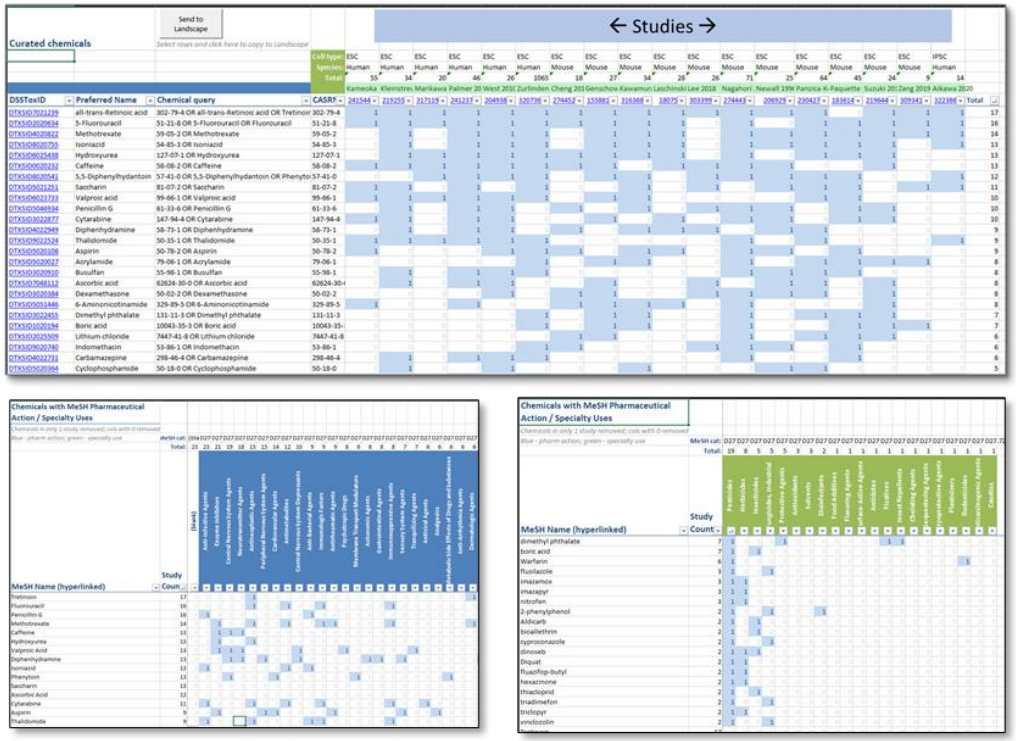
Funding: our research with human pluripotent stem cell lines (hPSCs) was performed under EPA's *Chemical Safety for Sustainability Research Program, Research Area 5 'Virtual Tissue Models' (VTMs).*

Compliance*:* CSS work involving established hPSC lines is compliant with Executive Order 13505 (issued 2009) to ensure that is ethically responsible, scientifically worthy, and conducted in accordance with applicable law.

Embryonic PSC lines are registered in the NIH Human Embryonic Stem Cell Registry: WA09 (H9) NIH Approval Number NIHhESC-10-0062 (EPA contract EP-D-13-055 with Stemina Biomarker Discovery) and RUES2, NIH Approval Number: NIHhESC-09-0013.

Other induced PSC lines: endodermal hPSC line from Allele Biotech #ABPSC-HDFAIPS (EPA contract EP-D-13-054 with Vala Sciences, Inc.).

Conceptual and practical considerations



Abstract Sifter, SWIFT, MeSH terms, Chemicals Dashboard, …

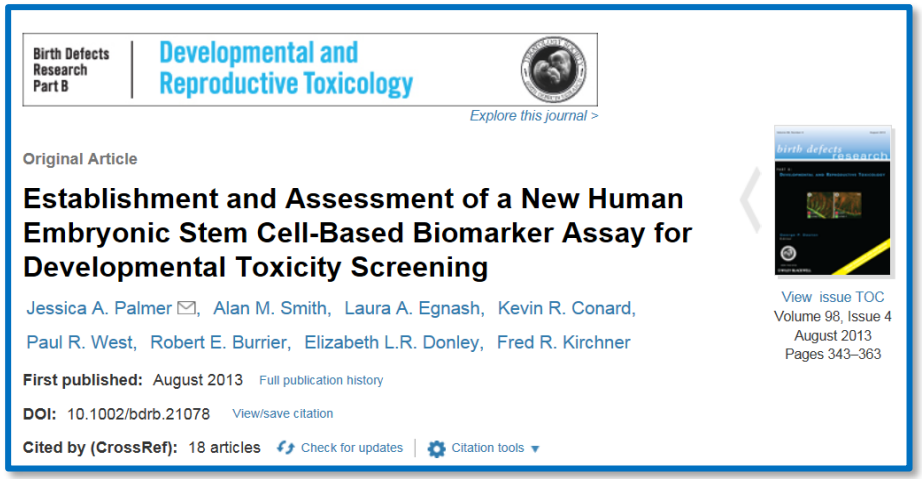
- **Detailed literature review:** survey of extant ESC assays used to classify developmental toxicants:
  - chemical domain
  - biological domain
  - standardized protocols
  - reproducibility
  - biomarker readouts
  - predictive power.

1,533 records in PubMed reduced to 333 (AI for relevance) and 192 (manual curation).

- **1,250 annotated chemicals (through 2020):**
  - 18 publications tested ≥ 10 compounds (primary)
  - 174 publications tested 1-9 (evidentiary support)
  - most frequently represented: ATRA, 5-FU, MTX.

Piersma et al., manuscript in final preparation

devTOX^{qP} assay: *Stemina Biomarker Discovery, EPA contract EP-D-13-055*



Birth Defects Research Part B | Developmental and Reproductive Toxicology

Explore this journal >

Original Article

Establishment and Assessment of a New Human Embryonic Stem Cell-Based Biomarker Assay for Developmental Toxicity Screening

Jessica A. Palmer ✉, Alan M. Smith, Laura A. Egnash, Kevin R. Conard, Paul R. West, Robert E. Burrier, Elizabeth L.R. Donley, Fred R. Kirchner

First published: August 2013 Full publication history

DOI: 10.1002/bdrb.21078 View/save citation

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View issue TOC
Volume 98, Issue 4
August 2013
Pages 343–363

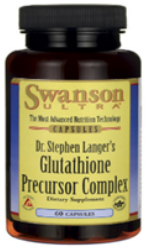
Pluripotent H9 hESC metabolomics assay "… identified the potential developmental toxicants in the test set with 77% accuracy (57% sensitivity, 100% specificity)."

Palmer et al. (2013) Birth Defects Res

Ornithine release
urea cycle, polyamine & pyrimidine synthesis.

TI = ORN/CYSS

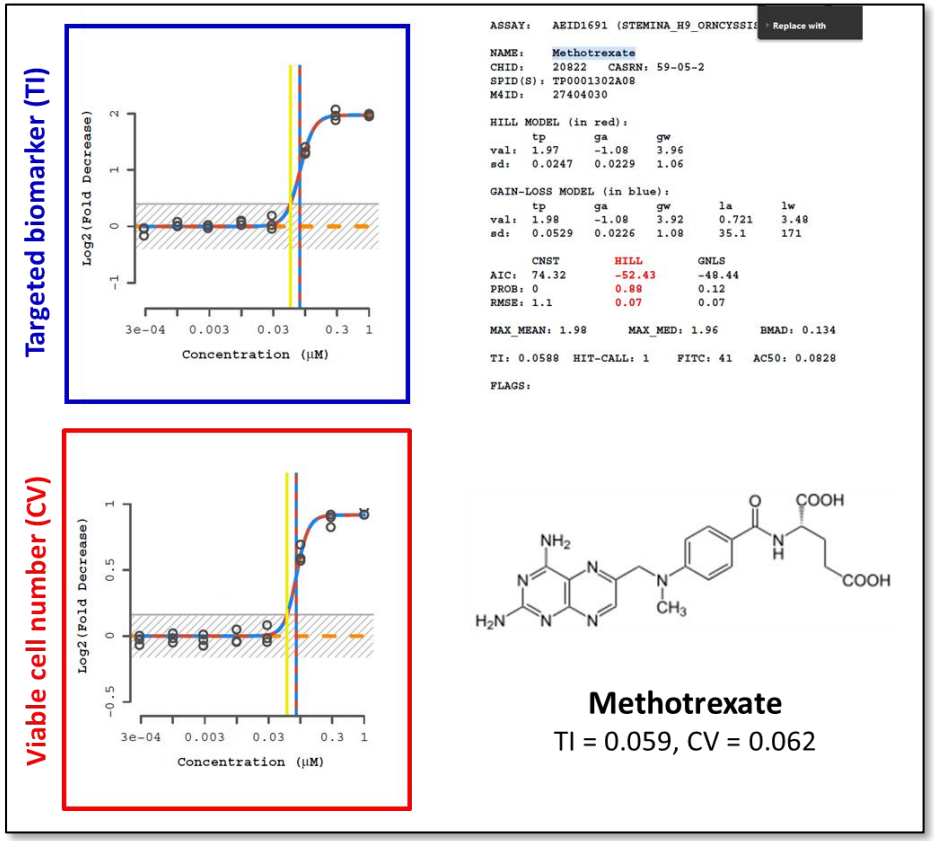
Cystine utilization
glutathione synthesis, redox cycling.



devTOX^{qP} profiling of the ToxCast chemical library

- 1065 ToxCast Ph I/II chemicals at single-conc. or multi-conc.;
- data pipelined to *in vitro-db_v3* database (>1125 features);
- ToxCast_STM dataset includes controls for data quality;
- Dataset now available in EPA's CompTox Chemicals Dashboard.

https://comptox.epa.gov/dashboard

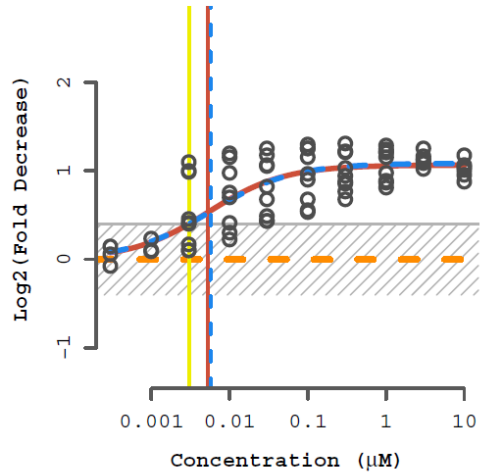


Zurlinden et al. (2020) Toxicol Sci

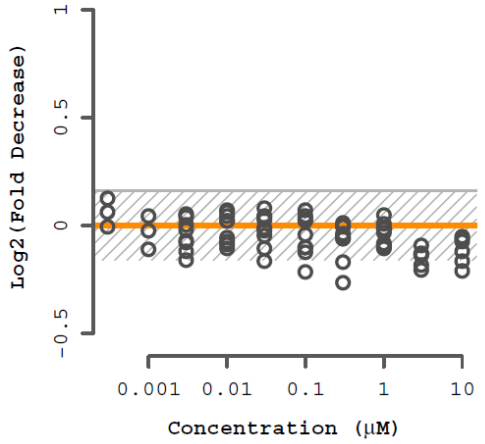
19.2% positive response rate indicative of teratogenic potential

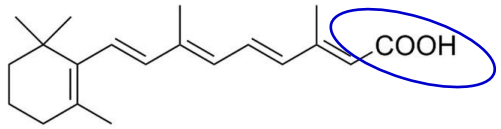
Example 1: *vitamin-A and its morphogenetic metabolite (all-trans Retinoic acid)*

Targeted biomarker (TI)



Viable cell number (CV)



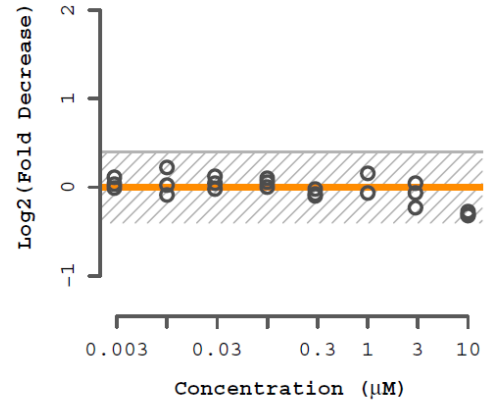


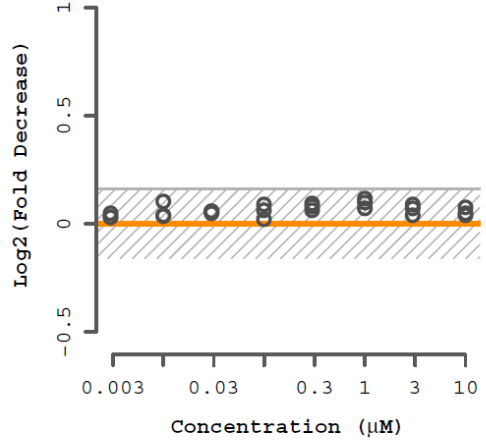
all trans Retinoic acid

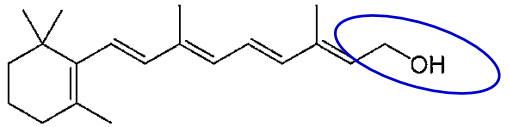
TI = 0.003 µM, CV = NA

dLEL rat = 2.5 mg/kg/day

dLEL rabbit = 0.5 mg/kg/day







Retinol (vitamin-A)

TI = NA, CV = NA

(True Negative)

Molecular characterization of a toxicological tipping point:

transcriptomic (RNAseq) signature of adaptation versus adversity

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Molecular characterization of a toxicological tipping point during human stem cell differentiation

Katerine S. Saili[a], Todor Antonijevic[a,b,c], Todd J. Zurlinden[a], Imran Shah[a], Chad Deisenroth[a], Thomas B. Knudsen[a,*]

ARTICLE INFO

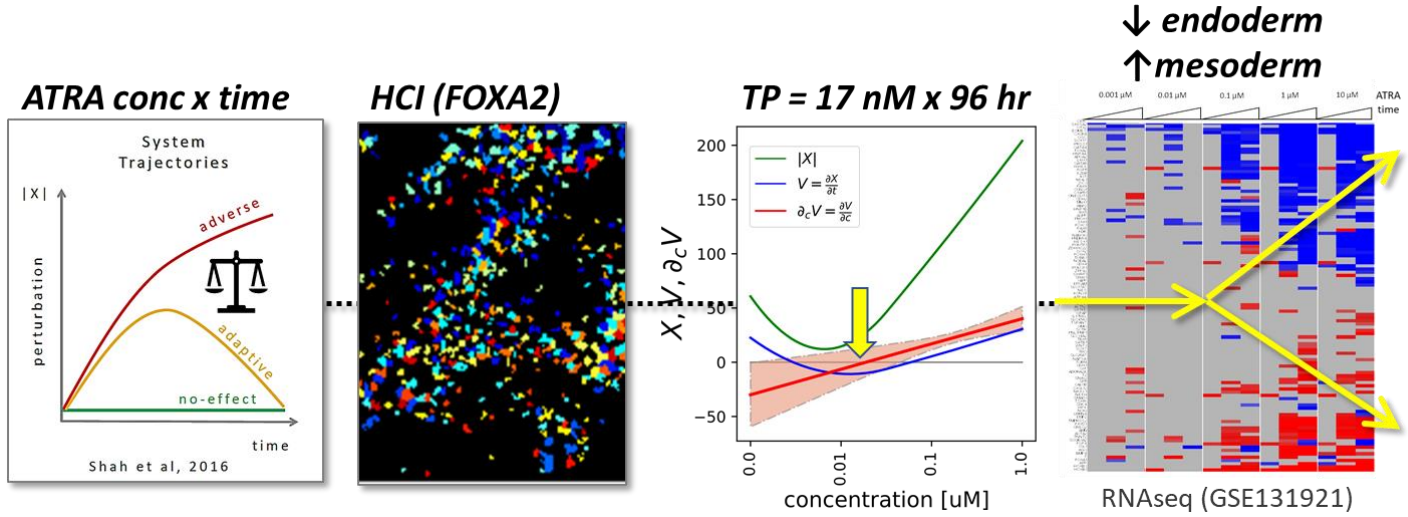
ABSTRACT

1. Introduction

Saili et al. (2020) Reprod Tox

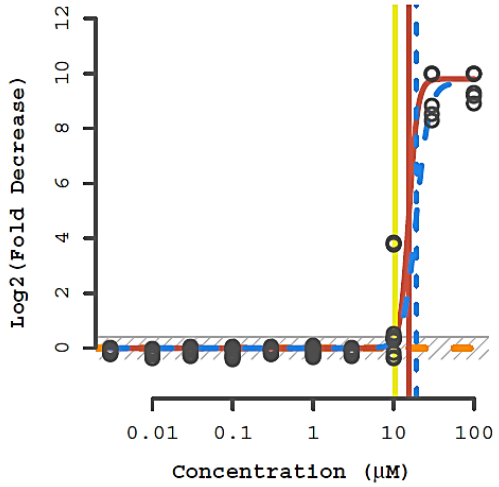
- What would a 'toxicological tipping point' on hPSC differentiation look like at the molecular level? Model = all trans retinoic acid (ATRA).
- ATRA is an endogenous signal (< 10 nM) and human teratogen (> 30 nM); tipping point computed at 17 nM @ 96 hr by imaging FOXA2 biomarker.
- RNAseq showed dysregulation by *EOMES* that normally drives endodermal specification and mesodermal delamination during gastrulation.

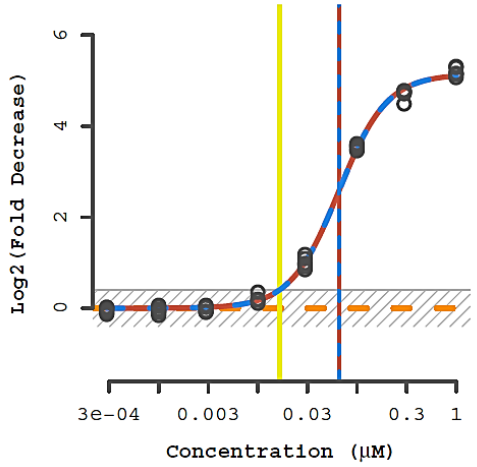
Awarded best paper of the year in *Reproductive Toxicology*



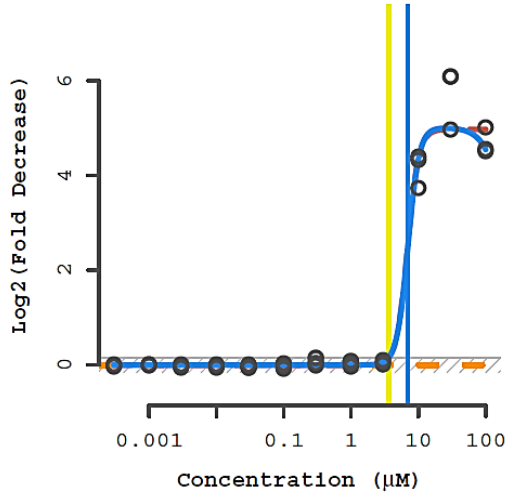
Example 2: *pharmacological angiogenesis inhibitors*

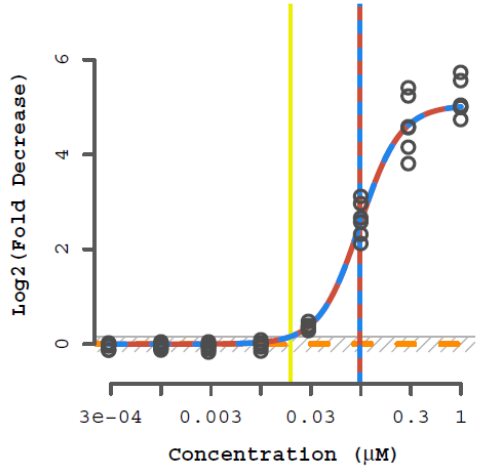
Targeted biomarker (TI)

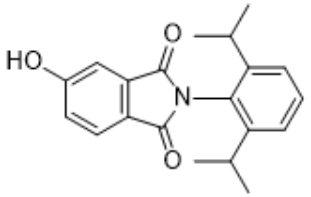




Viable cell number (CV)





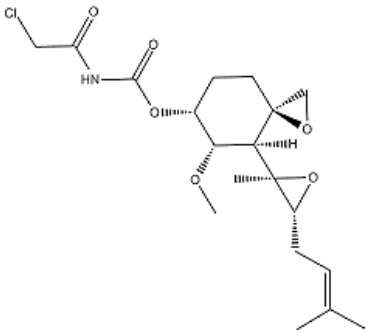


synthetic thalidomide analog

5HPP-33

TI = 10.5, CV = 16.4

(no rat or rabbit data)



synthetic fumagillin analog

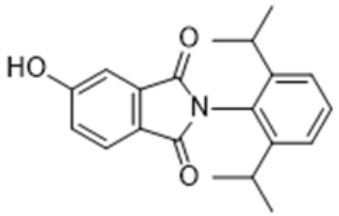
TNP-470

TI = 0.017, CV = 0.020

(no rat or rabbit data)

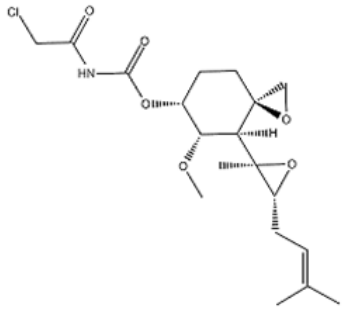
Case study: *checking forward predictivity of the hPSC assay*

Colleagues at Dow Chemical, led by Ed Carney, tested T.I. predictions for two structurally diverse potential vascular disrupters (pVDCs) in rat whole embryo culture (WEC):



5HPP-33: *synthetic thalidomide analog*

- T.I. predicted by hESC 10.5 µM
- AC50 observed in WEC 21.2 µM (embryo viability)



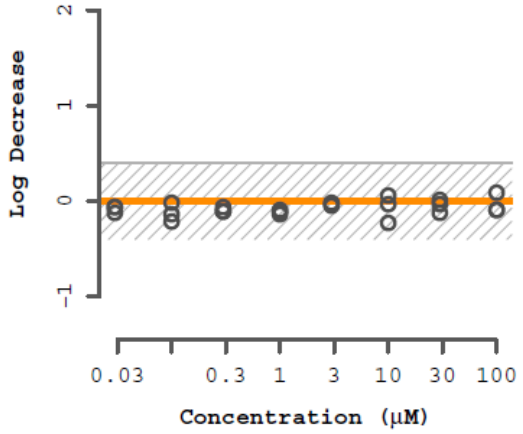
TNP-470: *synthetic fumagillin analog*

- T.I. predicted by hESC 0.02 µM
- AC50 observed in WEC 0.04 µM (dysmorphogenesis)

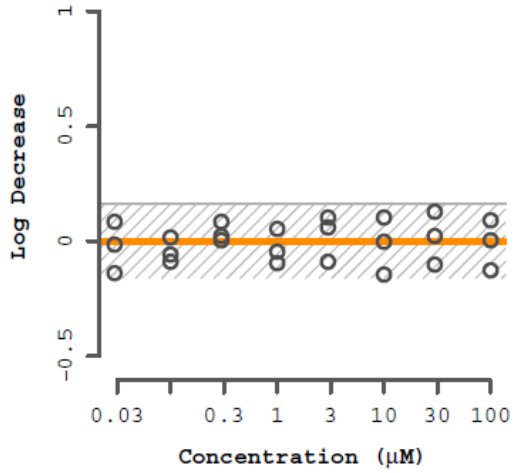
Ellis-Hutchings et al. (2017) Reprod Toxicol

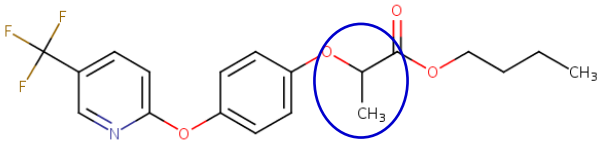
Example 3: *R-enantiomer (Fluazifop-P-butyl) is the active herbicide*

Targeted biomarker (TI)



Viable cell number (CV)



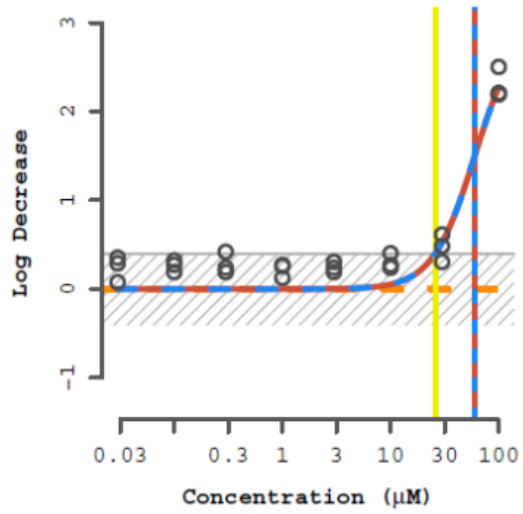


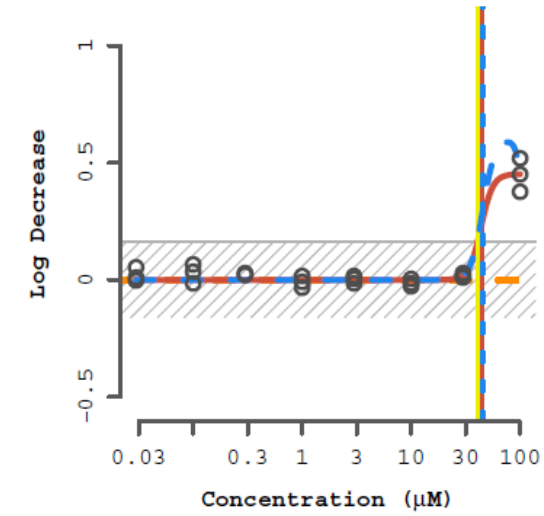
Fluazifop butyl

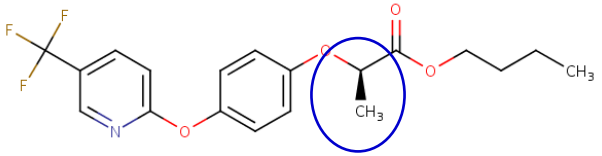
TI = not active, CV = no effect

dLEL rat = 10 mg/kg/day (< mLEL)

dLEL rabbit = 90 mg/kg/day (mLEL)







Fluazifop-P-butyl

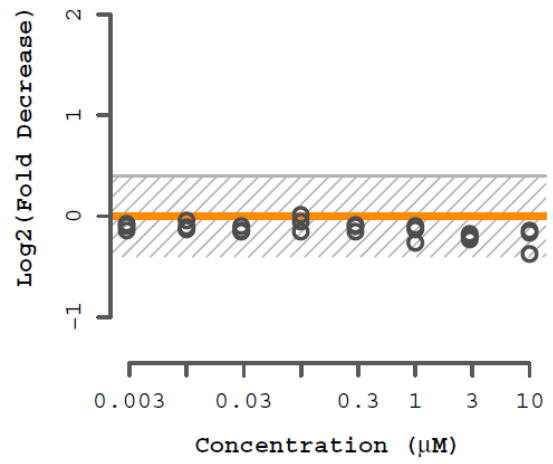
TI = 26 µM, CV = 40.8 µM

dLEL rat = 5 mg/kg/day (< mLEL)

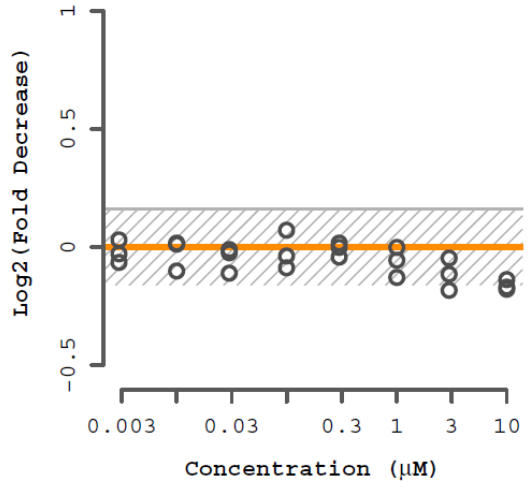
dLEL rabbit = 50 mg/kg/day (mLEL)

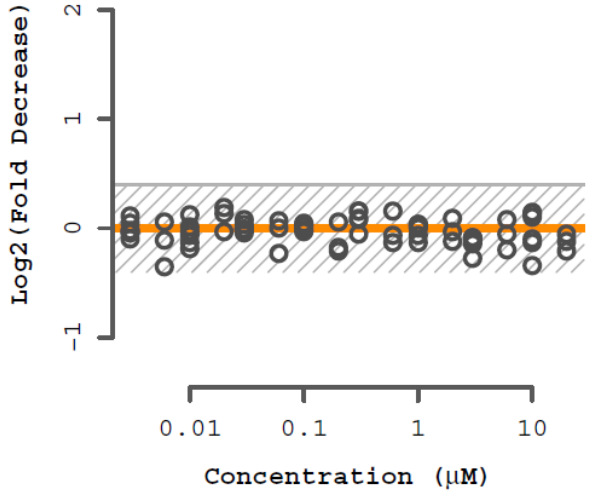
Example 4: *false negatives (not detected in ToxCast_STM)*

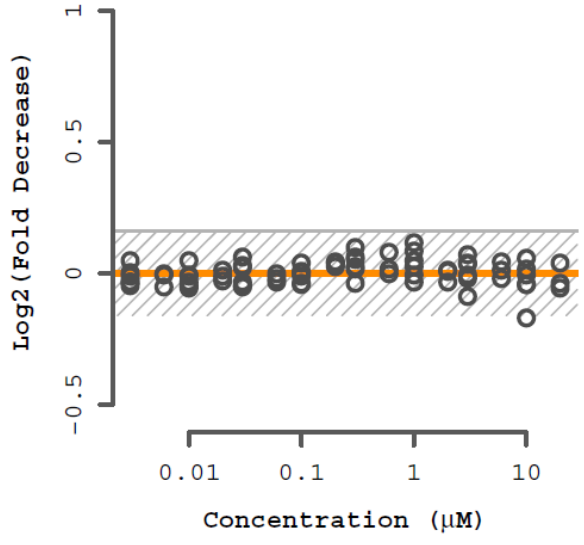
Targeted biomarker (TI)

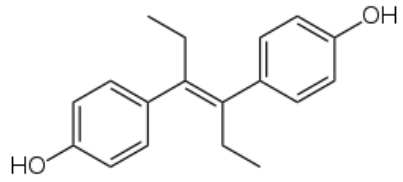


Viable cell number (CV)







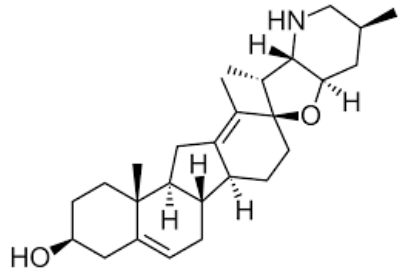


Diethylstilbestrol (DES)

TI = NA, CV = NA

dLEL rat = 0.03 mg/kg/day (= mLEL)

(no rabbit data in ToxRefDB)

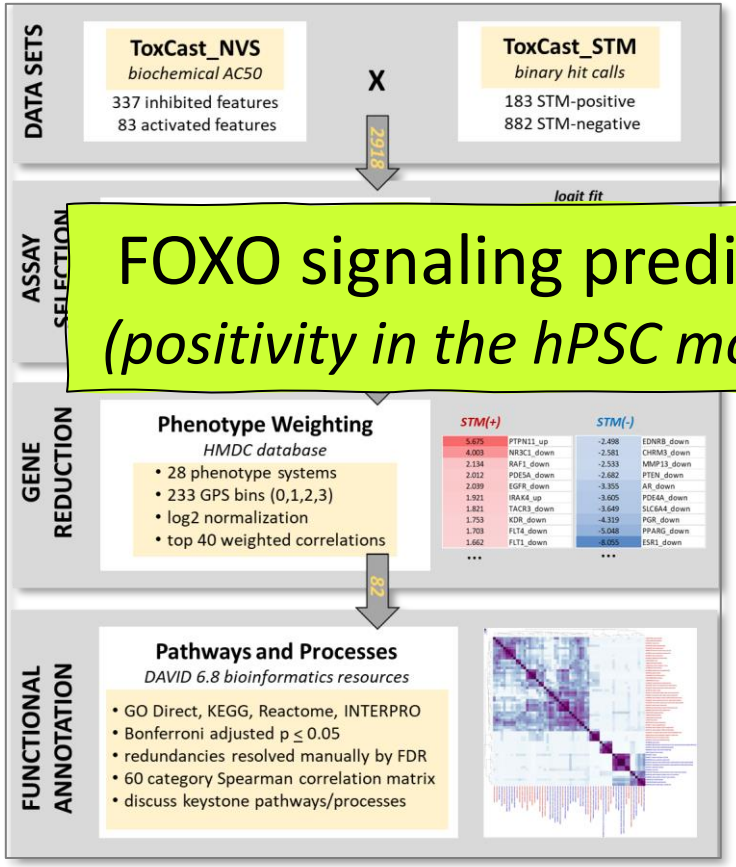


Cyclopamine

TI = NA, CV = NA

What human relevant pathways are detected or missed?

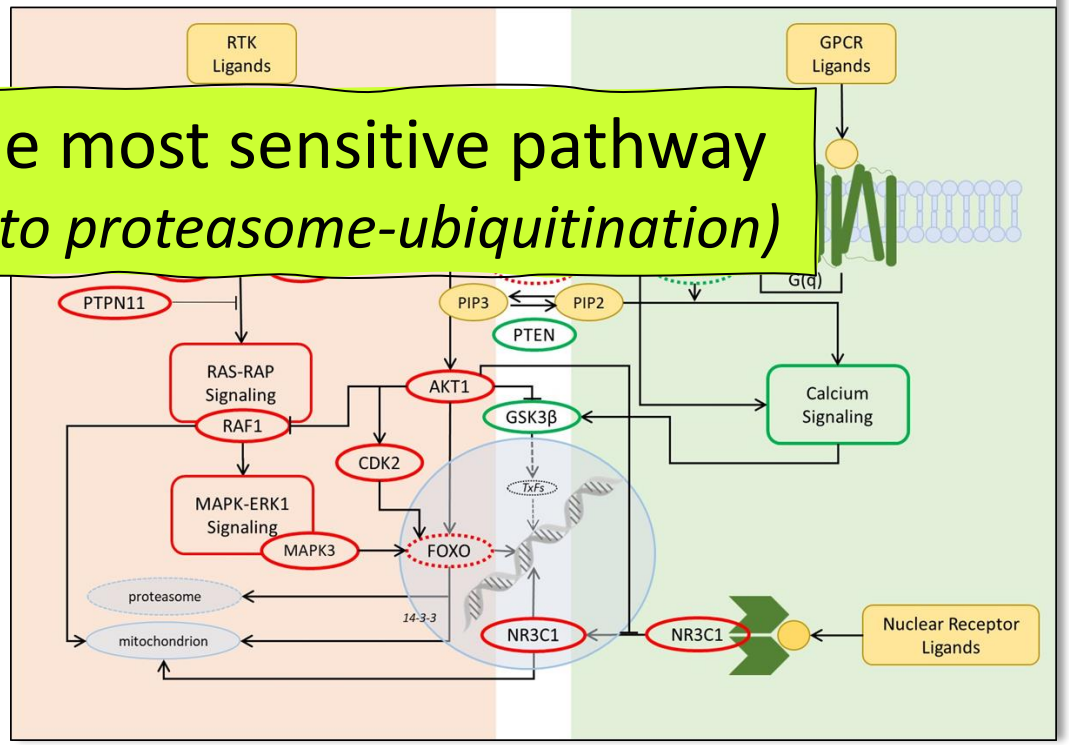
Workflow to mine the hPSC model against 337 biochemical assays in the ToxCast_NVS platform



FOXO signaling predicted as the most sensitive pathway
(positivity in the hPSC model driven to proteasome-ubiquitination)

Sensitive Domain **Insensitive Domain**

Annotation System	Keystone Pathway / Process	# MIEs	Class
GOTERM_BP_DIRECT	GO:0014066~regulation of phosphatidylinositol 3-kinase signaling	6	TP
KEGG_PATHWAY	hsa04068:FoxO signaling pathway	8	TP
KEGG_PATHWAY	hsa04510:Focal adhesion	13	TP
GOTERM_BP_DIRECT	GO:0007200~phospholipase C-activating G-protein coupled receptor signaling pathway	10	FN
INTERPRO	IPR001723:Steroid hormone receptor	7	FN
GOTERM_MF_DIRECT	GO:0005496~steroid binding	5	FN



Zurlinden et al. (2020) Toxicol Sci

Performance check for hPSC-based classification of DevTox

- Qualification on 42 well-curated reference compounds often used to validate alternative DevTox platforms[1].
- Balanced Accuracy (BAC) = 82% (0.65 sensitivity, 1.00 specificity) for these reference chemicals.
- Metrics are consistent with the original pharma-trained model [Palmer et al. 2013].

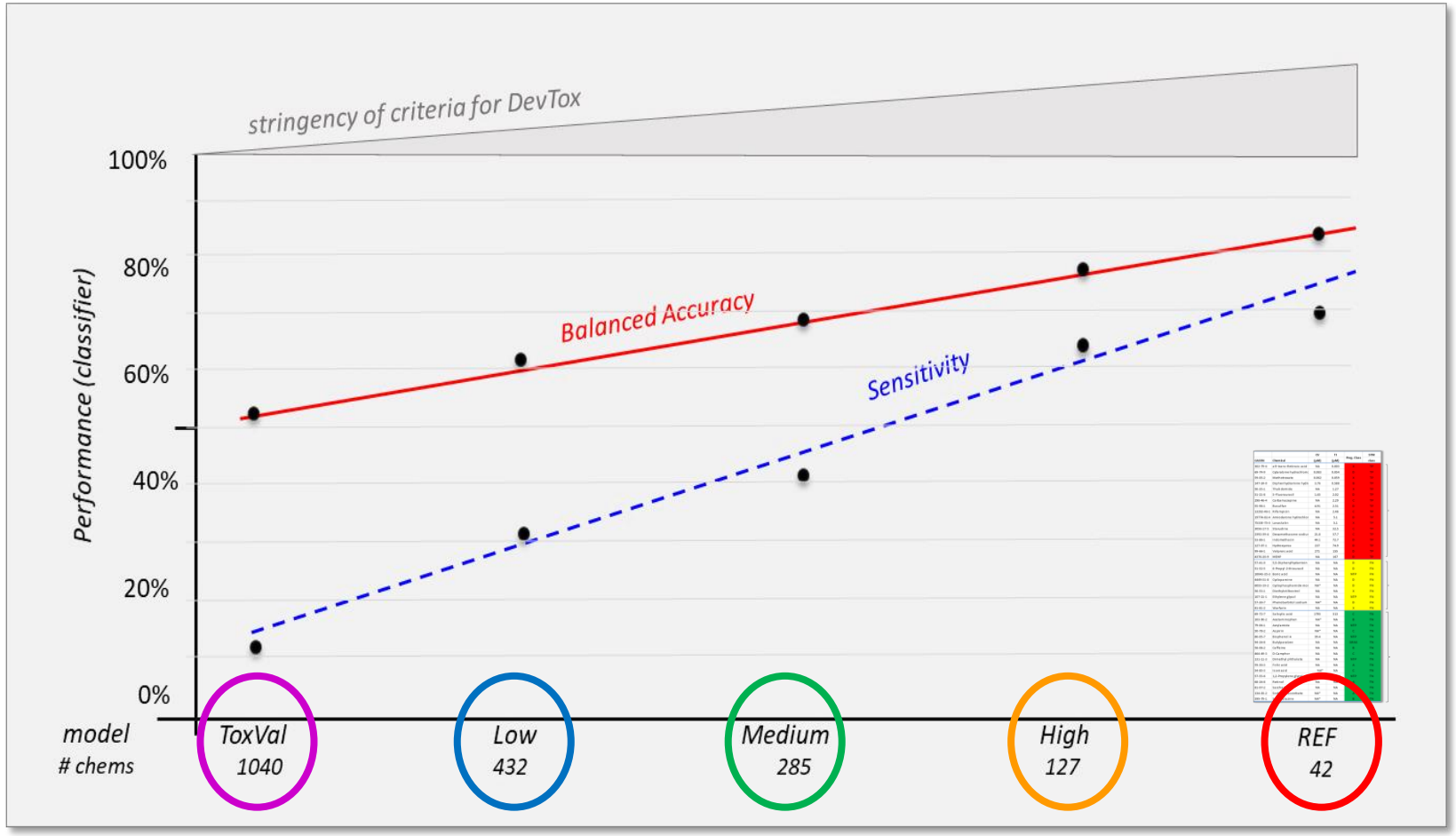
Many alternative assays have been validated with a limited set of data-rich chemicals, inflating predictive capacity of >80%; this has hampered regulatory acceptance.

CASRN	Chemical	CV (µM)	TI (µM)	Preg. Class	STM class	
302-79-4	all-trans-Retinoic acid	NA	0.003	X	TP	True Positive
69-74-9	Cytarabine hydrochloric	0.083	0.054	D	TP	
59-05-2	Methotrexate	0.062	0.059	X	TP	
147-24-0	Diphenhydramine hydr	3.76	0.588	B	TP	
50-35-1	Thalidomide	NA	1.27	X	TP	
51-21-8	5-Fluorouracil	1.45	2.02	D	TP	
298-46-4	Carbamazepine	NA	2.29	C	TP	
55-98-1	Busulfan	4.91	2.31	D	TP	
13292-46-1	Rifampicin	NA	2.46	C	TP	
19774-82-4	Amiodarone hydrochlor	NA	5.1	D	TP	
75330-75-5	Lovastatin	NA	5.1	X	TP	
3056-17-5	Stavudine	NA	32.5	C	TP	
2392-39-4	Dexamethasone sodiur	21.8	37.7	C	TP	
53-86-1	Indomethacin	44.1	72.7	D	TP	
127-07-1	Hydroxyurea	237	74.9	D	TP	
99-66-1	Valproic acid	271	155	D	TP	
4376-20-9	MEHP	NA	167	D	TP	
57-41-0	5,5-Diphenylhydantoin	NA	NA	D	FN	False Negative
51-52-5	6-Propyl-2-thiouracil	NA	NA	D	FN	
10043-35-3	Boric acid	NA	NA	NTP	FN	
4449-51-8	Cyclopamine	NA	NA	D	FN	
6055-19-2	Cyclophosphamide mor	NA*	NA	D	FN	
56-53-1	Diethylstilbestrol	NA	NA	X	FN	
107-21-1	Ethylene glycol	NA	NA	NTP	FN	
57-30-7	Phenobarbitol sodium	NA*	NA	D	FN	
81-81-2	Warfarin	NA	NA	X	FN	
69-72-7	Salicylic acid	1795	513	C	TN	True Negative
103-90-2	Acetaminophen	NA*	NA	B	TN	
79-06-1	Acrylamide	NA	NA	NTP	TN	
50-78-2	Aspirin	NA*	NA	C	TN	
80-05-7	Bisphenol A	39.4	NA	NTP	TN	
94-26-8	Butylparaben	NA	NA	GRAS	TN	
58-08-2	Caffeine	NA	NA	B	TN	
464-49-3	D-Camphor	NA	NA	C	TN	
131-11-3	Dimethyl phthalate	NA	NA	NTP	TN	
59-30-3	Folic acid	NA	NA	A	TN	
54-85-3	Isoniazid	NA*	NA	C	TN	
57-55-6	1,2-Propylene glycol	327552	246664	NTP	TN	
68-26-8	Retinol	NA	NA	A	TN	
81-07-2	Saccharin	NA	NA	A	TN	
134-03-2	Sodium L-ascorbate	NA*	NA	A	TN	
599-79-1	Sulfasalazine	NA*	NA	B	TN	

Zurlinden et al. (2020), Toxicol Sci

[1] Genschow et al. 2002; West et al. 2010; Daston et al. 2014; Augustine-Rauch et al. 2016; Wise et al. 2016

Chemical landscape: *hPSC biomarker (in vitro) and ToxRefDB (in vivo)*



Scaling Criteria (ToxRefDB)

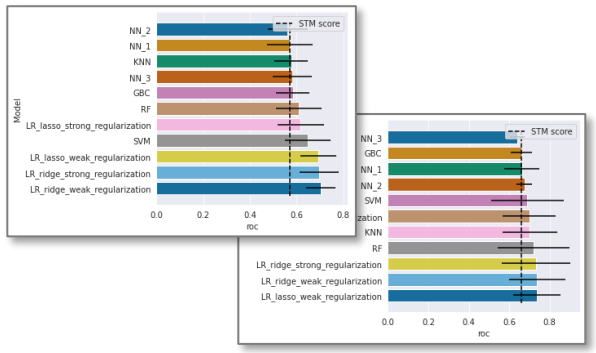
- **BM-42 reference**
- **concordant, rat AND rabbit**
- **dLEL < mLEL, rat OR rabbit**
- **dLEL ≤ 200 mg/kg/day**
- **LEL for any study type**

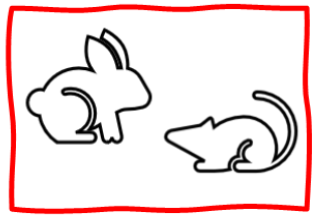
Predictivity of the hPSC biomarker declined as fetal outcome gained less concordance between rat-rabbit and concurrent maternal toxicity.

Zurlinden et al. (2020), Toxcol Sci

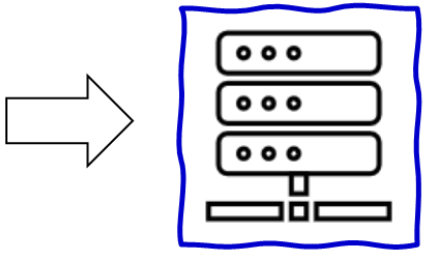
Bridging animal-human studies

- Although positivity rate (19.2%) of the hPSC assay was similar to concordant rat-rabbit studies (18.7%), only a subset of positives was detected by both platforms;
- challenge for holistic understanding of the applicability domain and blind spots of *in vitro* platforms, as well as mechanisms against which bioactivity data may be qualified.



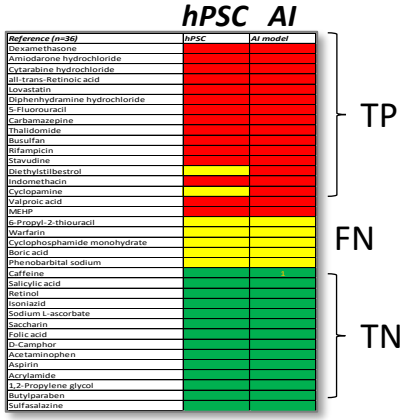


Data-driven model



Mechanistic model

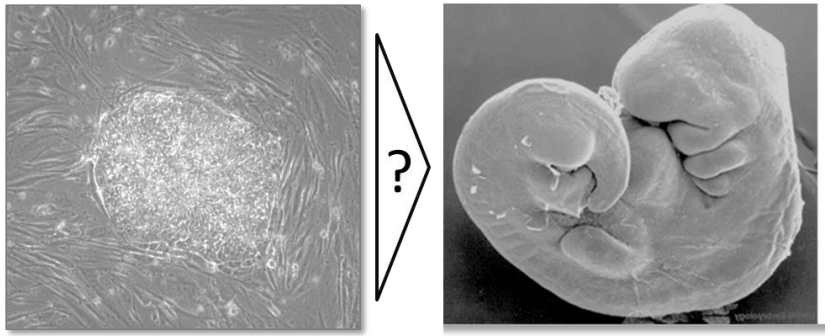
Forward prediction



Preliminary: AI model built solely from *in vitro* readouts (~1125 features) improved sensitivity over the hPSC biomarker alone (BAC = 86.8% vs 83.3%), but still misses a few.

Zurlinden et al., manuscript in preparation

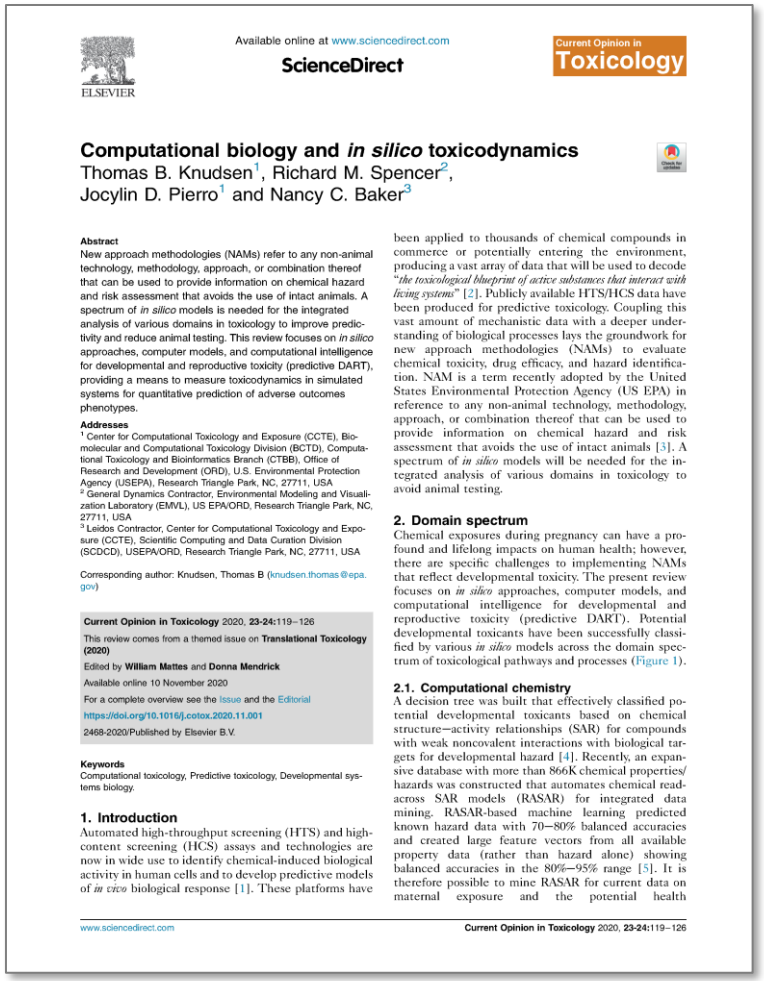
Can a hPSC assay live up to the NAM challenge?



Motivation for a building a more synoptic view to improve mechanistic understanding of developmental processes and toxicities around hPSCs.

- does not encompass the full complexity of anatomical development;
- blind to the precise spatial-temporal control of cell-cell interactions *in vivo* ;
- misses developmental effects secondary to maternal or placental toxicity;
- uncertainty of post-organogenesis vulnerability and post-natal manifestations;
- cross-species extrapolation (mESC to human, hPSC to animals);
- limited xenobiotic metabolism and other ADME considerations (toxicokinetics);
- uncertainties in translatability to the intact embryo (toxicodynamics).

2. A more synoptic view …



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Current Opinion in Toxicology

Computational biology and *in silico* toxicodynamics

Thomas B. Knudsen[1], Richard M. Spencer[2], Jocylin D. Pierro[1] and Nancy C. Baker[3]

Abstract
New approach methodologies (NAMs) refer to any non-animal technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals. A spectrum of *in silico* models is needed for the integrated analysis of various domains in toxicology to improve predictivity and reduce animal testing. This review focuses on *in silico* approaches, computer models, and computational intelligence for developmental and reproductive toxicity (predictive DART), providing a means to measure toxicodynamics in simulated systems for quantitative prediction of adverse outcomes phenotypes.

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2468-2020/Published by Elsevier B.V.

Keywords
Computational toxicology, Predictive toxicology, Developmental systems biology.

1. Introduction
Automated high-throughput screening (HTS) and high-content screening (HCS) assays and technologies are now in wide use to identify chemical-induced biological activity in human cells and to develop predictive models of *in vivo* biological response [1]. These platforms have been applied to thousands of chemical compounds in commerce or potentially entering the environment, producing a vast array of data that will be used to decode "*the toxicological blueprint of active substances that interact with living systems*" [2]. Publicly available HTS/HCS data have been produced for predictive toxicology. Coupling this vast amount of mechanistic data with a deeper understanding of biological processes lays the groundwork for new approach methodologies (NAMs) to evaluate chemical toxicity, drug efficacy, and hazard identification. NAM is a term recently adopted by the United States Environmental Protection Agency (US EPA) in reference to any non-animal technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals [3]. A spectrum of *in silico* models will be needed for the integrated analysis of various domains in toxicology to avoid animal testing.

2. Domain spectrum
Chemical exposures during pregnancy can have a profound and lifelong impacts on human health; however, there are specific challenges to implementing NAMs that reflect developmental toxicity. The present review focuses on *in silico* approaches, computer models, and computational intelligence for developmental and reproductive toxicity (predictive DART). Potential developmental toxicants have been successfully classified by various *in silico* models across the domain spectrum of toxicological pathways and processes (Figure 1).

2.1. Computational chemistry
A decision tree was built that effectively classified potential developmental toxicants based on chemical structure–activity relationships (SAR) for compounds with weak noncovalent interactions with biological targets for developmental hazard [4]. Recently, an expansive database with more than 866K chemical properties/hazards was constructed that automates chemical read-across SAR models (RASAR) for integrated data mining. RASAR-based machine learning predicted known hazard data with 70–80% balanced accuracies and created large feature vectors from all available property data (rather than hazard alone) showing balanced accuracies in the 80%–95% range [5]. It is therefore possible to mine RASAR for current data on maternal exposure and the potential health

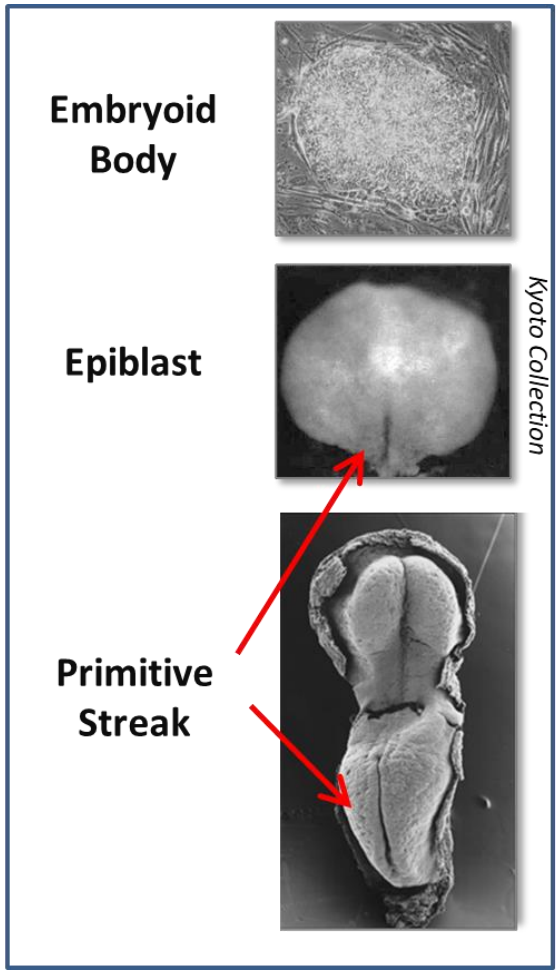
Knudsen et al. (2020) Curr Opin Toxicol

- Computational biology and computer simulation can extend data-driven models for mechanistic prediction.
- Enablers of virtual tissue models (VTMs):
  - **synthetic microsystems:** recapitulate the microphysiology, cellular behaviors and spatial dynamics of the physical system.
  - **computational intelligence:** biology-inspired algorithms use fuzzy logic to fill in missing or incomplete information.
  - **artificial life:** computer simulation of biological processes evolved through automation, control networks.

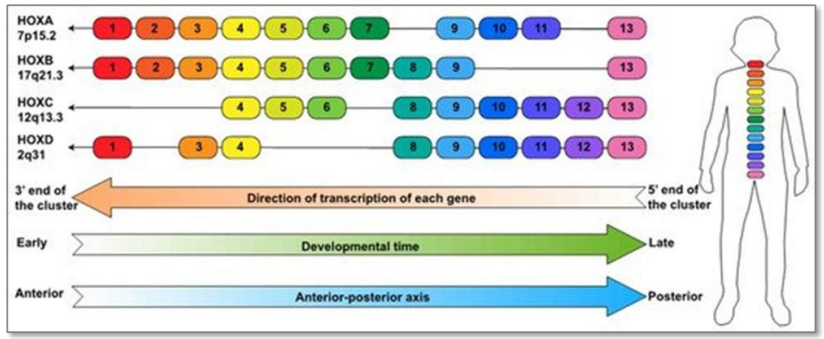
"Molecular biology took Humpty Dumpty apart … mathematical modeling is required to put him back together again." – Schnell et al. (2007) Amer Scientist

Gastrulating embryo:

remarkable example of a self-organizing system



- The molecular biology and behavior of hPSCs in culture most closely resembles the epiblast of an early embryo during 'gastrulation'.
- Gastrulation '*decodes the genomic blueprint of the fetal body plan* ' through complex signaling pathways (e.g, FOX, SOX, HOX).
- Cell migration through the primitive streak is essential for regional organization but cultured hPSCs lack this positional information.

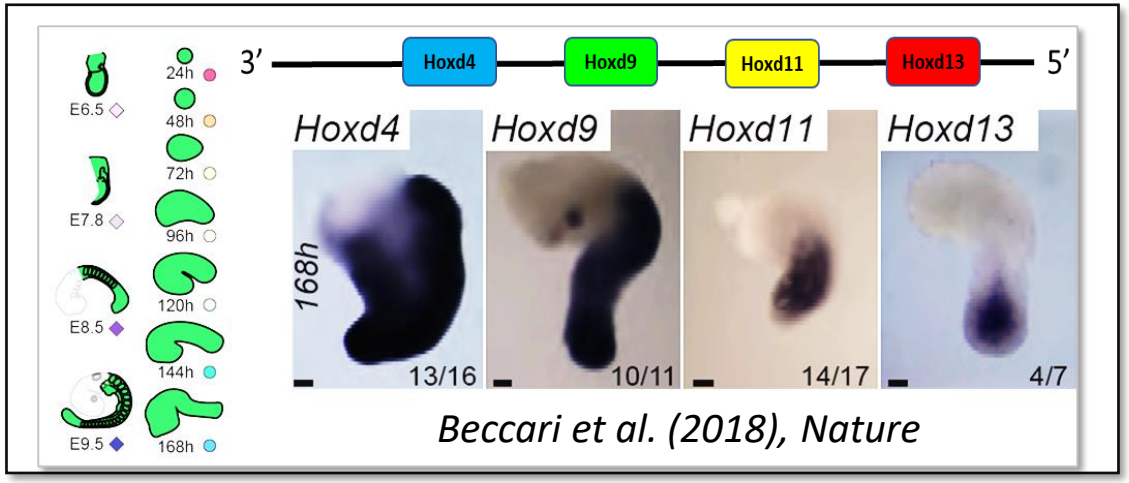


Luo et al. (2019)

"It is not birth, marriage, or death, but **gastrulation** which is truly the most important time in your life."

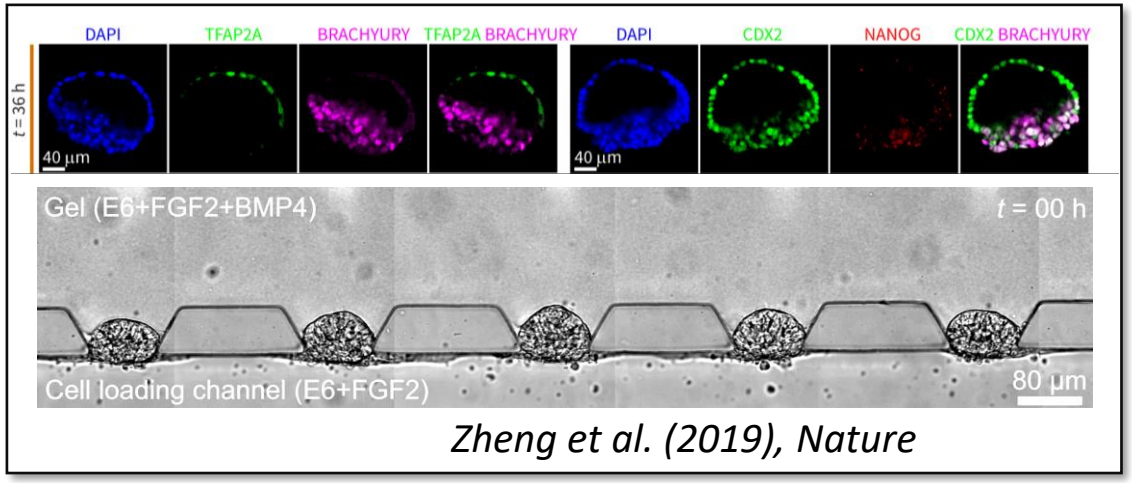
- Lewis Wolpert

Engineered *in vitro* microsystems



Beccari et al. (2018), Nature

- iPSC-derived microsystems can self-organize at least some positional information.
- **Example**: colinear *Hox* expression in 'gastruloids' forming from mESC-aggregate.



Zheng et al. (2019), Nature

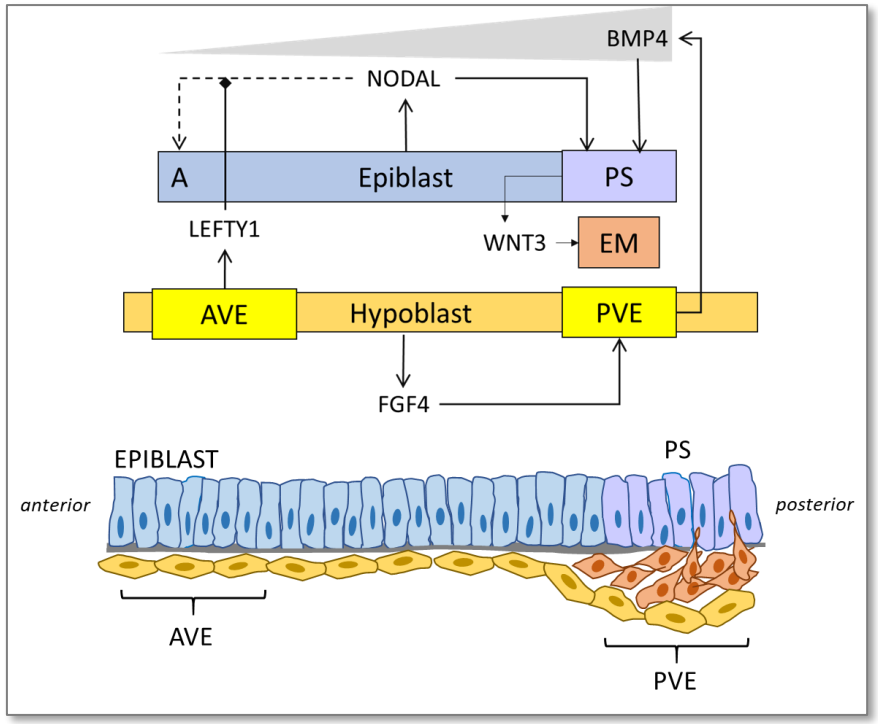
- Properties come naturally to the epiblast via positional cell-cell signaling.
- **Example:** restoring FGF2-BMP4 signaling polarizes a synthetic epiblast from hPSCs.

Computational (*in silico*) microsystems

Anatomical homeostasis in a self-regulating 'Virtual Embryo'

SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

Morphological programming logic of the epiblast



Agent-Based Models (ABMs)

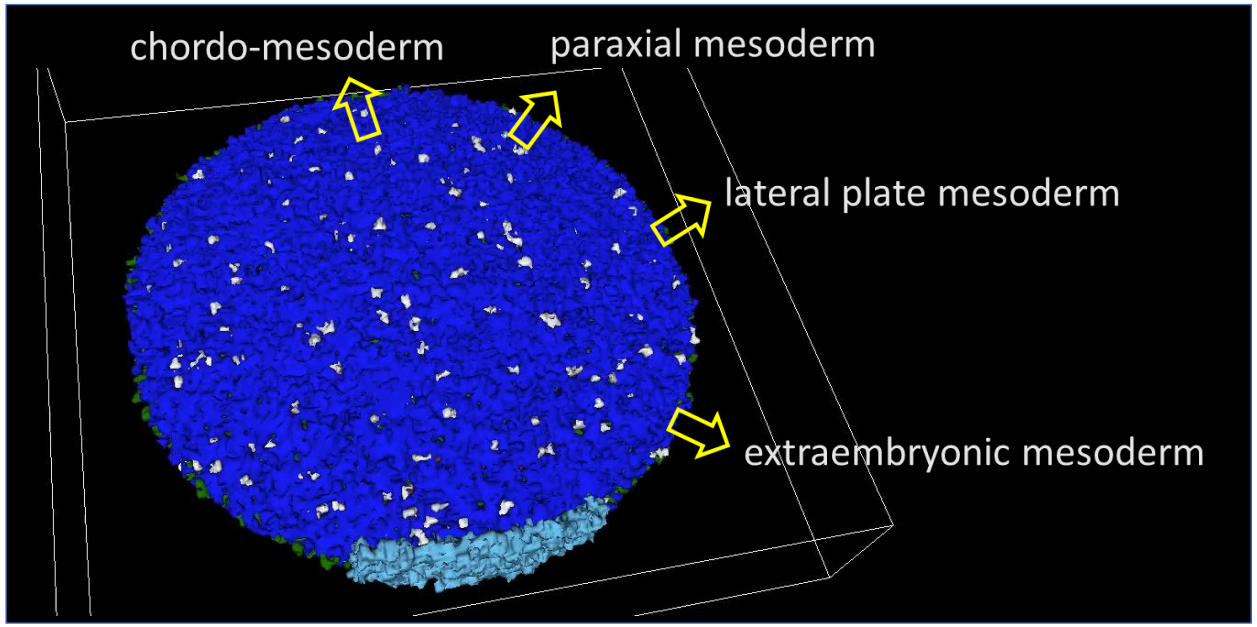
- nature-inspired *agents* (cells) and *rules* (behaviors) are set into motion as a self-organizing virtual system, using an open-source modeling environment (**CompuCell3d.org**).

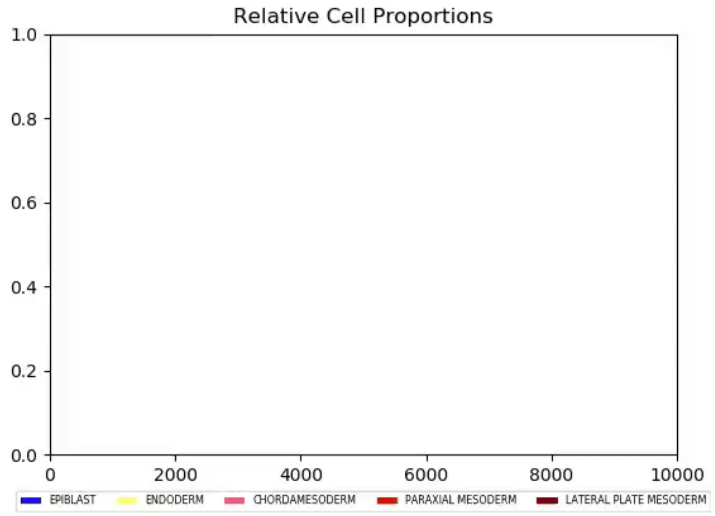
- soft-computing uses fuzzy logic to simulate forces or properties governing cell fate and behavior where rules are inexact or knowledge incomplete (**computational intelligence**).

- can change course in response to a particular situation or stimulus, such as genetic errors or biomolecular lesions introduced from real world data (**dynamic translation**).

- probabilistic rendering of where, when and how a particular condition might lead to an adverse developmental outcome (**cybermorphs**).

Quasi-gastrulation *in silico*



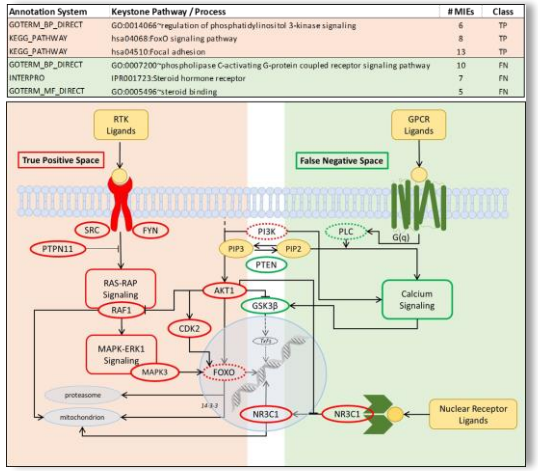


- Cellular systems agent-based model for the epiblast (ESABM) built in CompuCell3D.org to recapitulate cell movements and positional information.
- This virtual embryo model can be used to '*recode the genomic blueprint of the fetal body plan*' for *in silico* translation of hPSC chemical effects data.

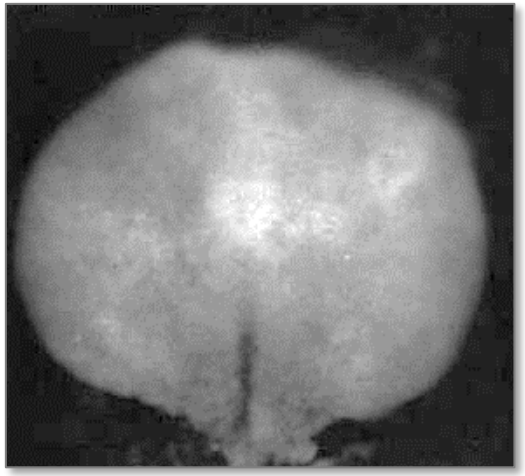
R Spencer, EMVL (work in progress

Synoptic manifold for toxicodynamics

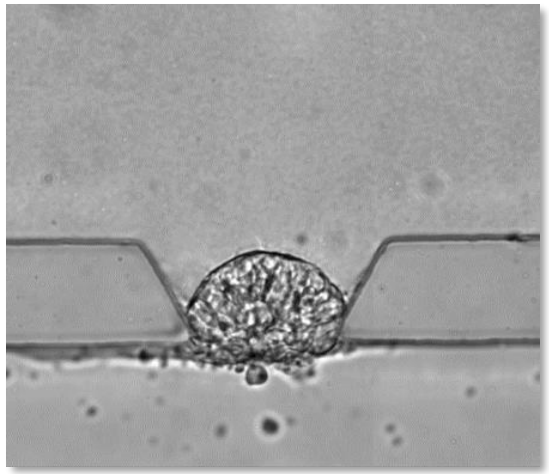
hPSC profiling



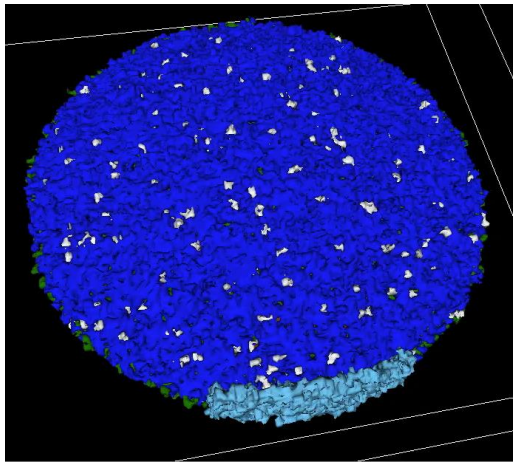
In vivo - knowledge



In vitro - MPS

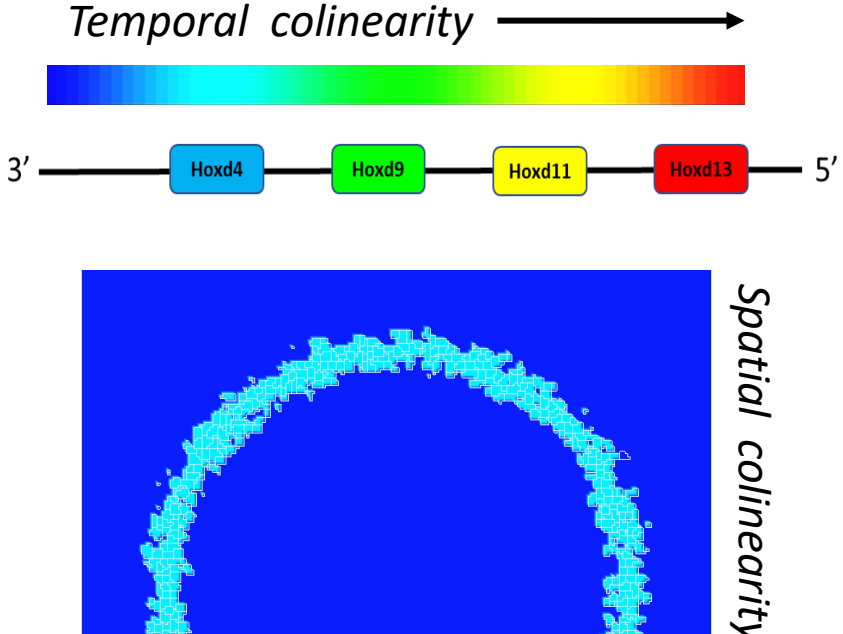


In silico - ABM



- **Bioactivity profiling:** high-throughput screening of hPSCs (e.g., ToxCast/Tox21)
- **Synthetic microsystems:** recapitulate the microphysiology of a physical system.
- **Computational intelligence:** fuzzy logic to fill in missing or incomplete information.
- **Artificial life:** biological plausibility evolved through automation, control networks.

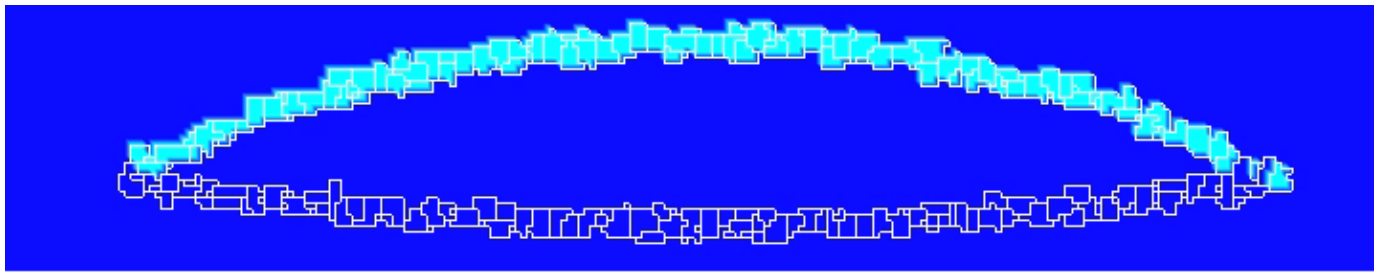
EXAMPLE: *perturbing the synthetic Hox clock (in silico)*



3500 migrating epiblast cells (5000 MCS)

R Spencer, EMVL (work in progress)

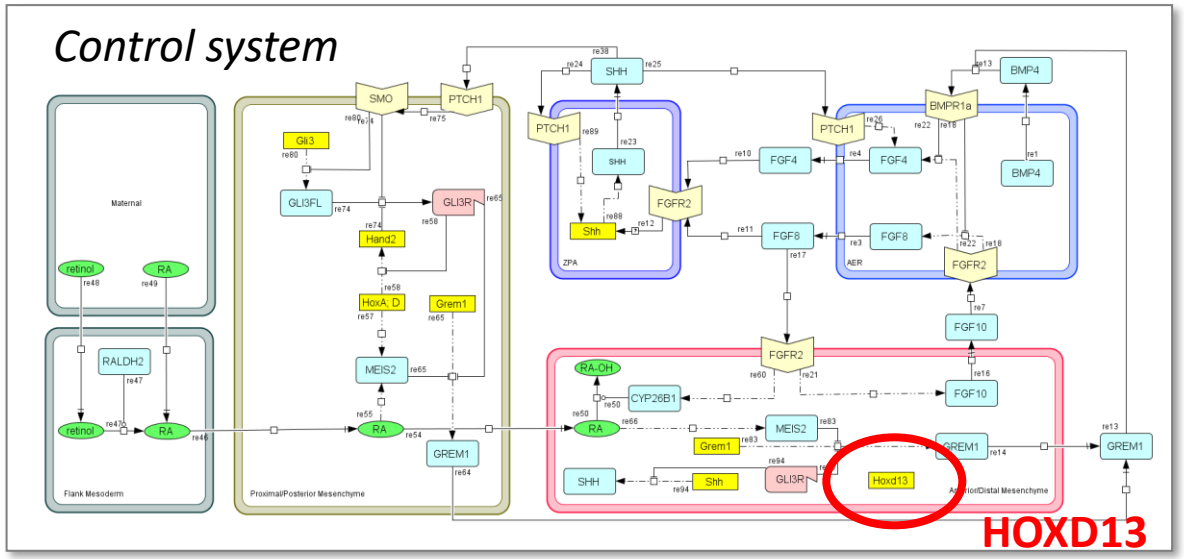
Transverse slice at the organizer node (4_9_11_13 @5000 MCS)

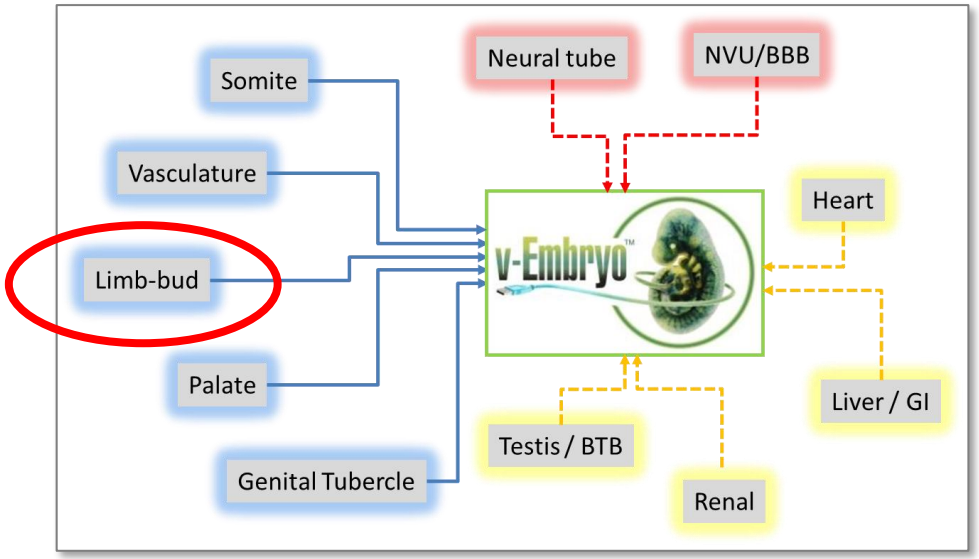


↓FGF signaling slows the Hox clock (4_9_11_13 @5000 MCS)

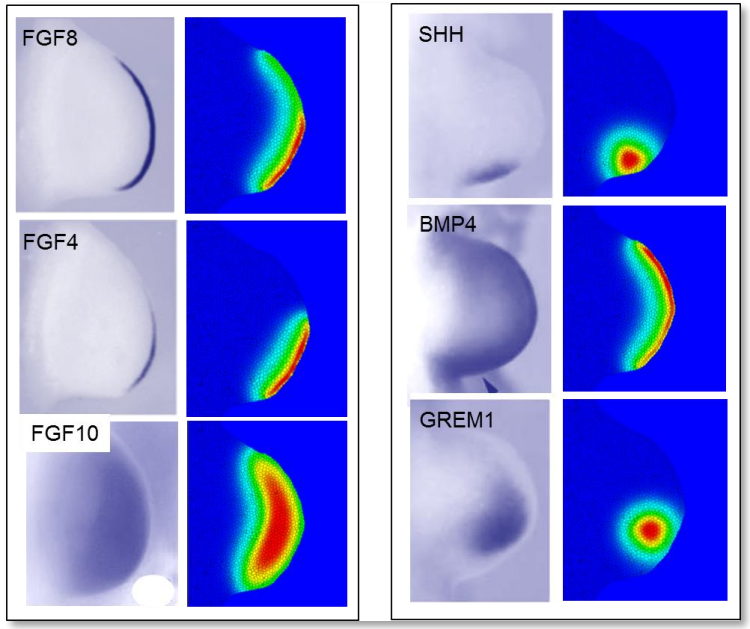
Deletions in the HOXD cluster that remove HOXD13 are associated with severe limb and genital defects

Example: *genetic regulation of early limb-bud development outgrowth*

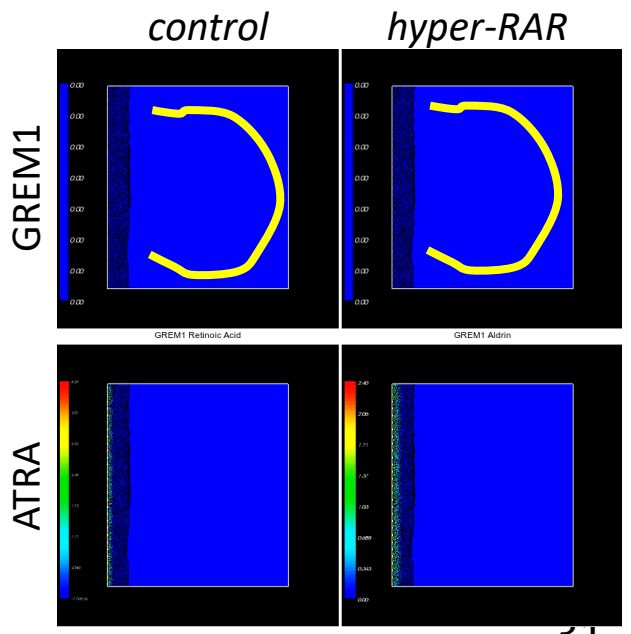




Simulated cell signal gradients encoded from gene expression (ISH)



Cybermorph foreshadows limb reduction defects following hyper-activation of RARs



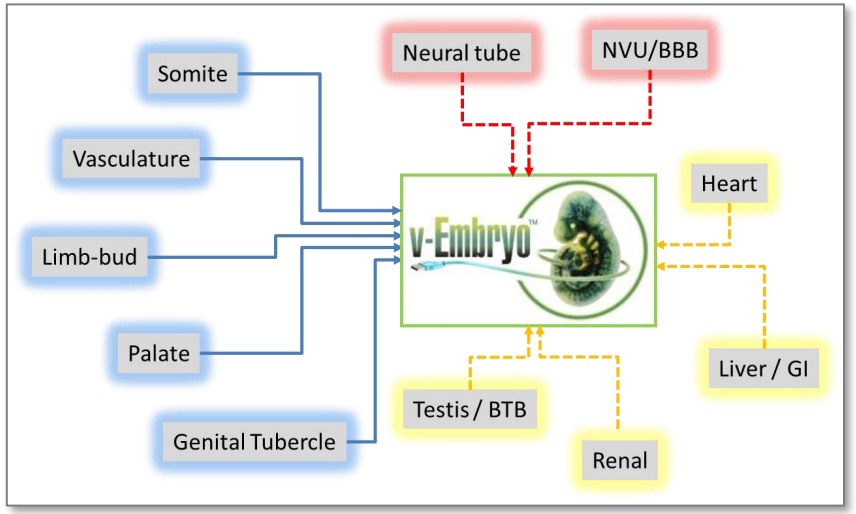
EPA/ORD/CCTE systems model, work in progress

Challenges for animal-free developmental prediction

Computational intelligence: *how complex must cellular systems models be for accurate phenotypic translation?*

Performance-based case studies: *what best practices are best suited for NAM implementation, circa 2025?*

Quantitative simulation*: how far can artificial life go towards replacing animal testing, circa 2035?*



Thank you!