



MASOT-MARTA
Ambassador series
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Predictive DART and Computational Intelligence: ***past, present, and future***

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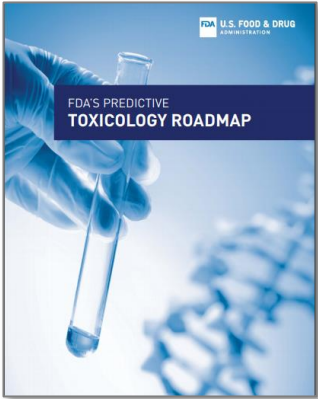
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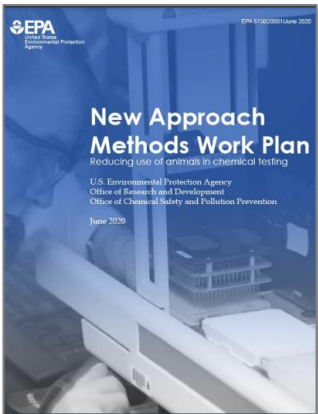
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Regulatory drivers



FDA's Predictive Toxicology Roadmap - created to identify the toxicology areas that could benefit from improved predictivity as well as promising new technologies that could potentially meet these needs and support animal 3Rs (Replacement, Reduction, and Refinement).

<https://www.fda.gov>



EPA's New Approach Methods Work Plan - created to prioritize agency efforts and resources toward activities that aim to reduce the use of animal testing while continuing to protect human health and the environment (NAMs).

<https://www.epa.gov>

Can the computer replace lab animal testing?

technology feature

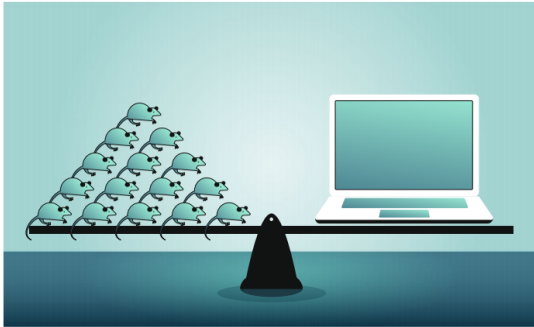
Toxicology testing steps towards computers

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 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

- **mapping the (chemical) world:** structural alerts based on black-box read-across [Structure-Activity Relationships].
- Luechtefeld et al. (2018) *Toxicol Sci* 165: 198-212.
- **opening the black box:** data-driven machine learning using high-throughput bioactivity datasets [ToxCast/Tox21].
- Ciallella and Zhu (2019) *Chem Res Toxicol* 32: 536-547.
- **a step further:** engineered microsystems (*in vitro*) and dynamic simulation (*in silico*) [Virtual Tissue Models].
- Knudsen et al. (2021) *Toxicol Sci* 180: 198-211.

Kling (2019) *Nature Lab Animal* 48: 40-42

DART translation: *in vitro* assays and *in silico* models that reflect embryo-fetal development and human pregnancy will be important for NAM-based evaluation of developmental hazard potential.

Pluripotent stem cell (PSC) assays

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

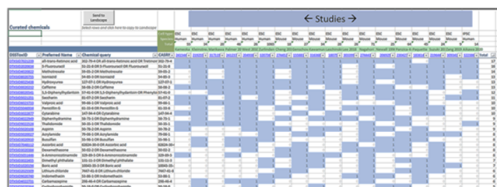
PSC assays in developmental toxicity

Detailed Review Paper (DRP) on EST platforms

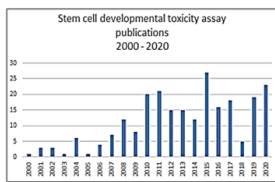


Writing team

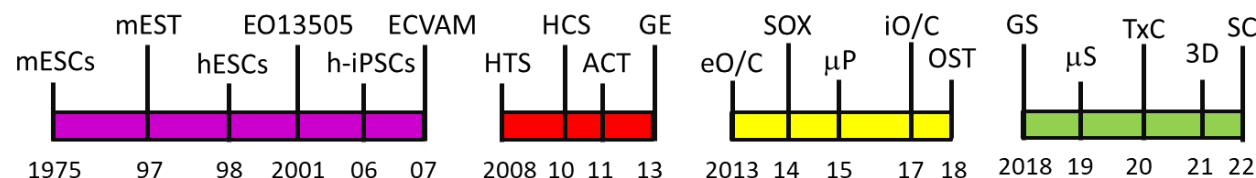
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 Aldert Piersma – RIVM (Netherlands)
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 Katya Tsaoun – Johns Hopkins University



July 18, 2019
 Systematic Scoping
 Literature Review Protocol for
 Embryonic Stem Cells In Vitro
 Developmental Toxicity Tests
 Authors: Nancy Baker, Hajime Kojima, Aldert Piersma, Horst Spielmann, Noriyuki Suzuki, Burkhard Flick, Michio Fujiwara, Thomas Knudsen, George Daston, Nancy Baker, Leidos



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



1997-07 traditional mEST [accuracy ~80%]

2008-13 improve mEST sensitivity and scalability [accuracy 72-83%]

2013-18 targeted biomarker readouts in hPSCs [accuracy 77-87+%]

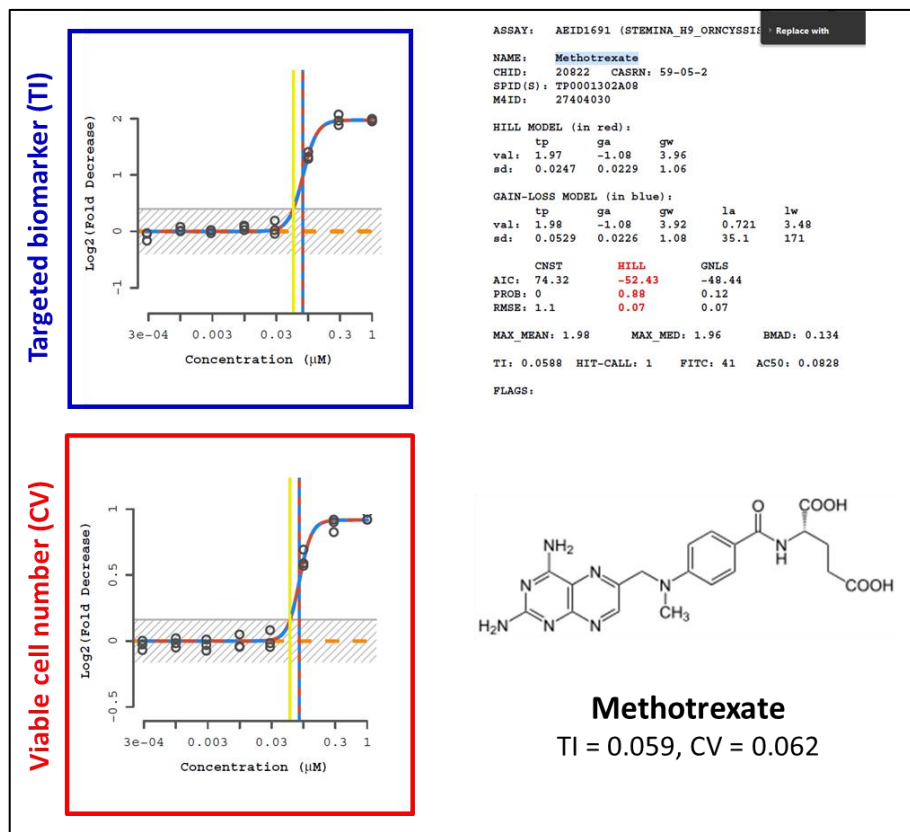
2018- hPSC biomimetics and throughput [accuracy 77-87+%]

- **Strategy:** search for studies that used PSCs to classify developmental toxicants:
 - chemical and biological domains
 - standardized protocols, biomarkers, readouts
 - reproducibility and performance.
- **Corpus:** 1,533 PubMed records (*circa 1991-2021*):
 - reduced to 192 papers by AI and manual curation
 - 18 papers tested ≥ 10 compounds (primary)
 - 174 papers tested 1-9 (evidentiary support).
- **1,250 annotated chemicals:**
 - accuracies 72-87% for well-curated compounds
 - most commonly represented: ATRA, 5-FU, MTX.

ToxCast_STM: *devTOX^{qP}* assay contracted from Stemina Biomarker Discovery

- 1065 ToxCast Ph I/II chemicals at single-conc. or multi-conc.;
- Data tcpl-pipelined into ToxCast database (now >1125 assays);
- Raw and pipelined data in EPA's CompTox Chemicals Dashboard.
- Bioactivity concentration predicting DevTox potential.

<https://comptox.epa.gov/dashboard>



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Research Article

SOT | Society of Toxicology
academic.oup.com/toxsci

Profiling the ToxCast Library With a Pluripotent Human (H9) Stem Cell Line-Based Biomarker Assay for Developmental Toxicity

Todd J. Zurlinden¹, Katherine S. Salli¹, Nathaniel Rush¹, Parth Kothiyar¹, Richard S. Judson¹, Keith A. Houck¹, E. Sidney Hunter¹, Nancy C. Baker¹, Jessica A. Palmer¹, Russell S. Thomas¹, and Thomas B. Knudsen^{1,*}

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ABSTRACT

The Stemina devTOX quickPredict platform is a human pluripotent stem cell-based assay that predicts the developmental toxicity potential based on changes in cellular metabolism following chemical exposure (Palmer, J. A., Smith, A. M., Egnash, L. A., Conard, K. R., West, P. R., Burrier, R. E., Donley, F. L. R., and Kirchner, F. R. (2013). Establishment and assessment of a new human embryonic stem cell-based biomarker assay for developmental toxicity screening. *BioTh Defect Res. B Dev. Reprod Toxicol.* 94, 343-361). Using this assay, we screened 1065 ToxCast phase I and II chemicals in single concentration or concentration-response for the targeted biomarker (ratio of ornithine to cystine secreted or consumed from the media). The dataset from the Stemina (STM) assay is annotated in the ToxCast portfolio as STM. Major findings from the analysis of ToxCast_STM dataset include (1) 19% of 1065 chemicals yielded a prediction of developmental toxicity, (2) assay performance reached 79%–82% accuracy with high specificity (> 84%) but modest sensitivity (< 67%) when compared with in vivo animal models of human prenatal developmental toxicity, (3) sensitivity improved as more stringent weights of evidence requirements were applied to the animal studies, and (4) statistical analysis of the most potent chemical hits on specific biochemical targets in ToxCast revealed positive and negative associations with the STM response, providing insights into the mechanistic underpinnings of the targeted endpoint and its biological domain. The results of this study will be useful to improving our ability to predict in vivo developmental toxicants based on in vitro data and in silico models.

Key words: predictive toxicology; developmental toxicity; embryonic stem cells.

In 2007, the National Research Council published *Toxicity Testing in the 21st Century: A Vision and a Strategy* (National Research Council, 2007). This report addressed the potential for automated high-throughput screening (HTS) and high-content screening (HCS) assays and technologies to identify chemically induced biological activity in human cells and to develop predictive models of in vivo biological response that would ignite a shift from traditional animal endpoint-based testing to human pathway-based risk assessment (Collins et al., 2008). Concurrent with the NRC 2007 report, the U.S. Environmental Protection

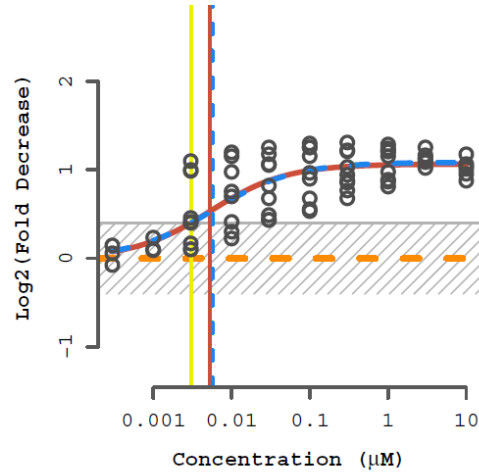
Published by Oxford University Press on behalf of the Society of Toxicology 2020. This work is written by US Government employees and is in the public domain in the US.

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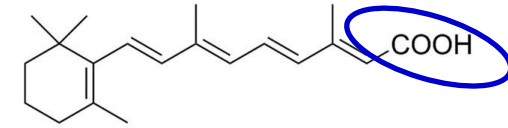
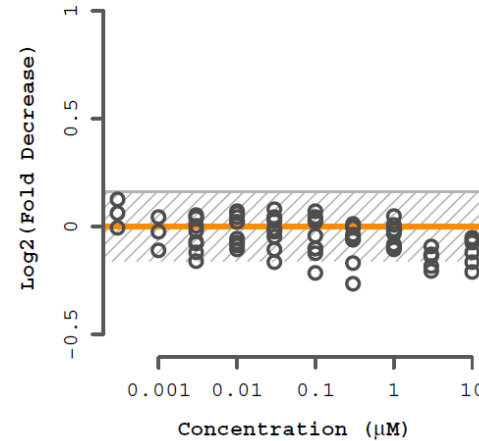
19.2% positivity rate indicative of teratogenic potential

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

Targeted biomarker (TI)



Viable cell number (CV)

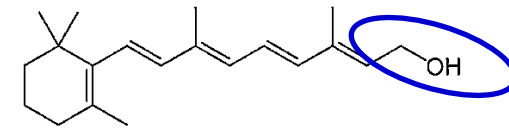
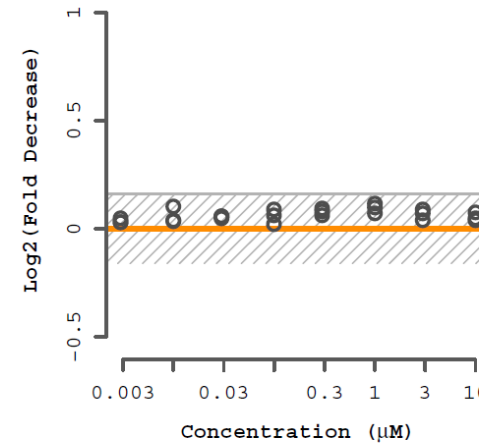
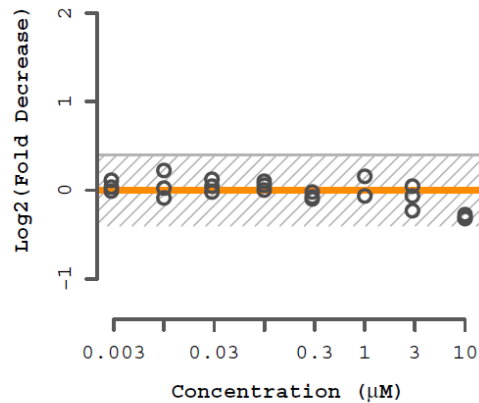


all trans Retinoic acid (ATRA)

TI = 0.003 μM, CV = NA

dLEL rat = 2.5 mg/kg/day

dLEL rabbit = 0.5 mg/kg/day



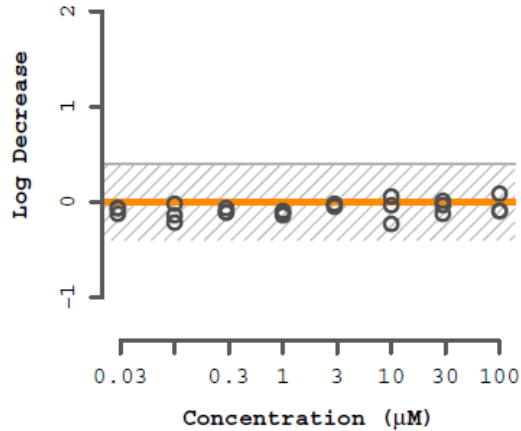
Retinol (vitamin-A)

TI = NA, CV = NA

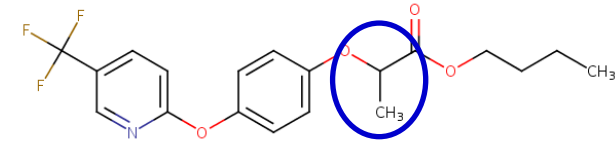
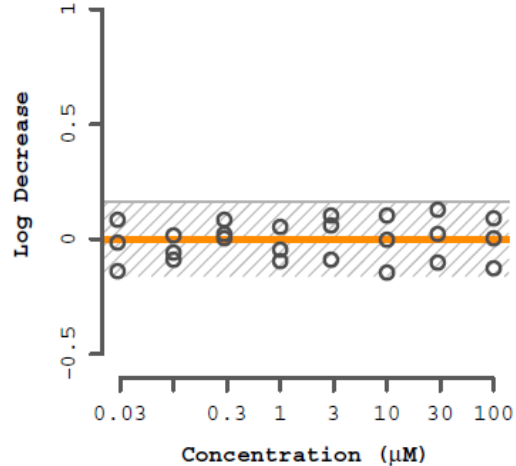
(True Negative)

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

Targeted biomarker (TI)



Viable cell number (CV)

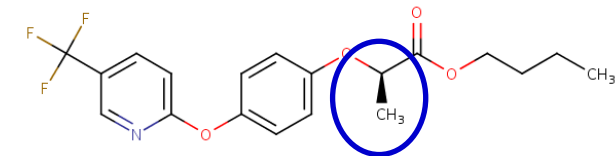
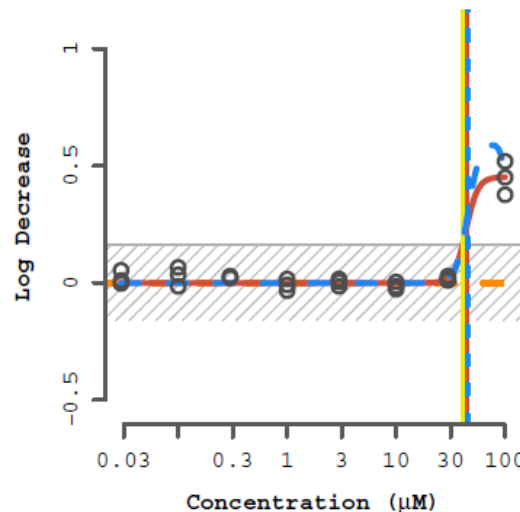
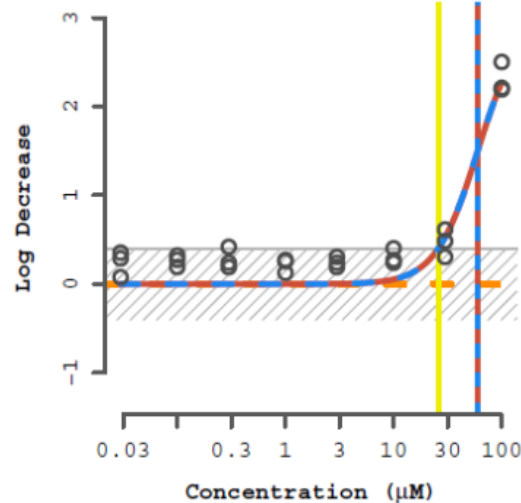


Fluazifop butyl

TI = not active, CV = no effect

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

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



Fluazifop-P-butyl

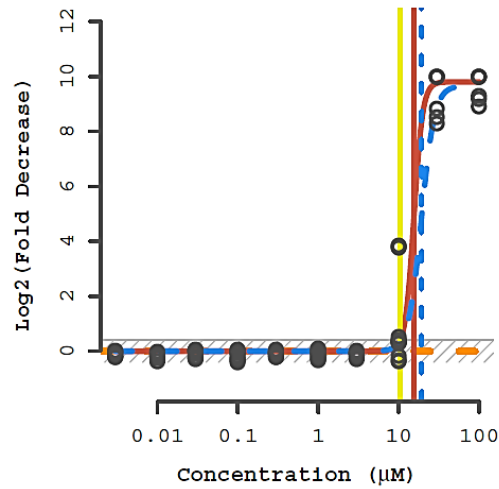
TI = 26 μM, CV = 40.8 μM

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

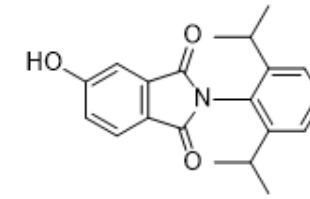
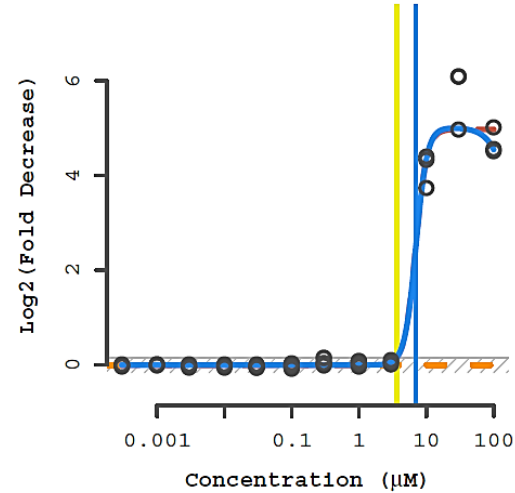
dLEL rabbit = 50 mg/kg/day (mLEL)

Example: *pharmacological angiogenesis inhibitors*

Targeted biomarker (TI)



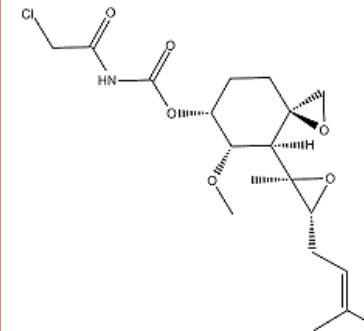
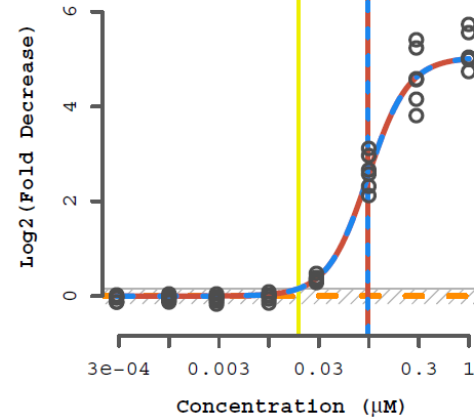
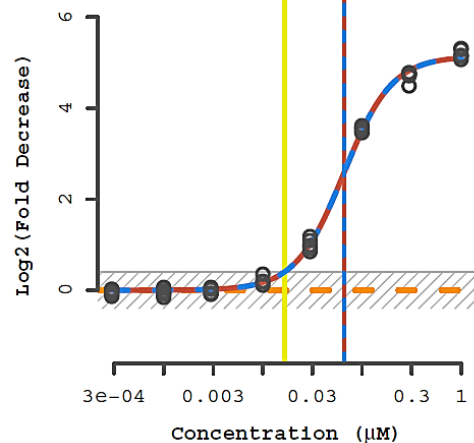
Viable cell number (CV)



synthetic thalidomide analog

5HPP-33

TI = 10.5, CV = 16.4
(no rat or rabbit data)



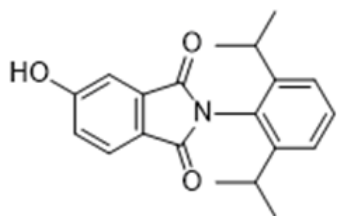
synthetic fumagillin analog

TNP-470

TI = 0.017, CV = 0.020
(no rat or rabbit data)

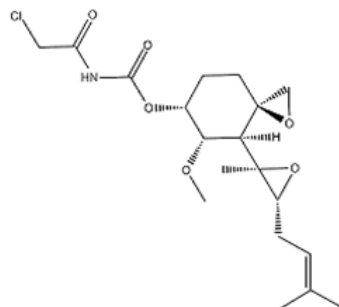
Quantitative prediction: *checking forward predictivity of the hPSC readout*

Colleagues at Dow Chemical, led by Ed Carney, tested T.I. predictions for two structurally diverse potential vascular disruptors (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)

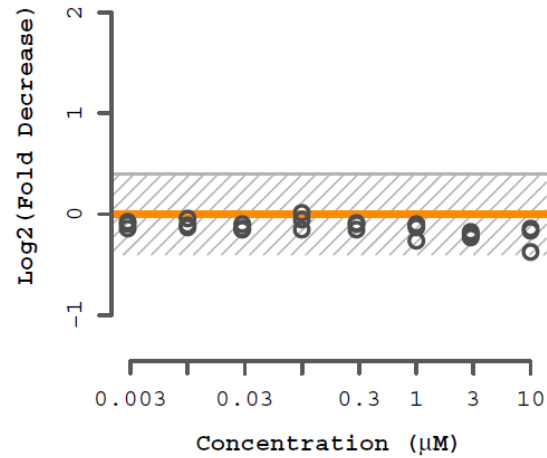


TNP-470: *synthetic fumagillin analog*

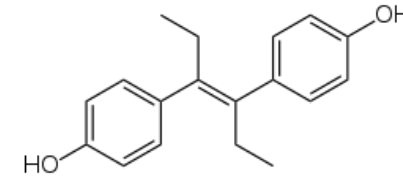
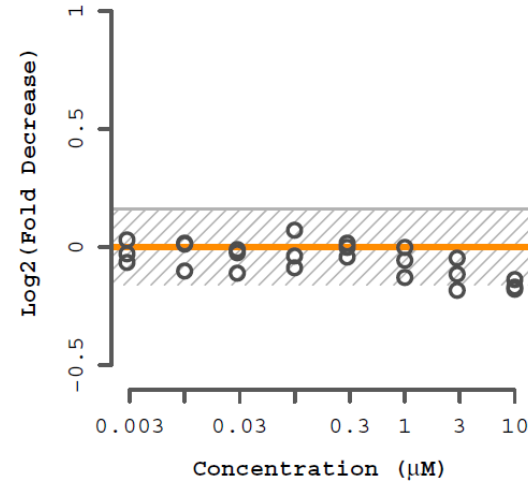
- T.I. predicted by hESC **0.02 μM**
- AC50 observed in WEC **0.04 μM** (dysmorphogenesis)

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

Targeted biomarker (TI)

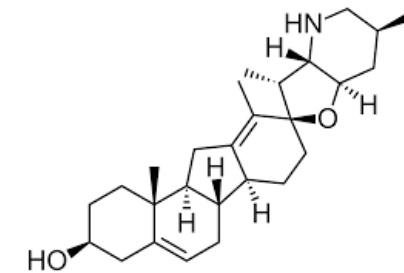
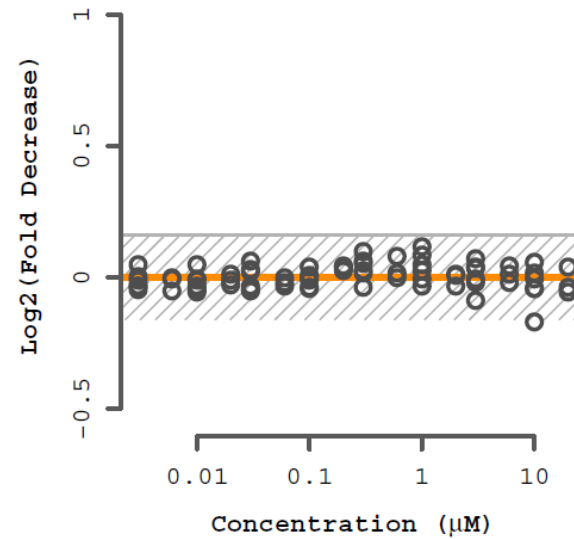
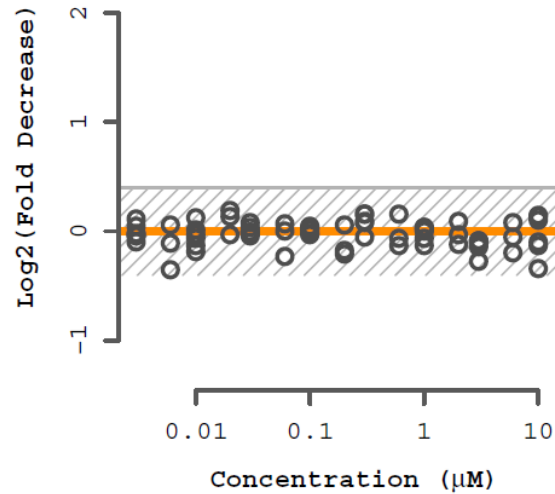


Viable cell number (CV)



Diethylstilbestrol (DES)

TI = NA, CV = NA
dLEL rat = 0.03 mg/kg/day (= mLEL)
(no rabbit data in ToxRefDB)

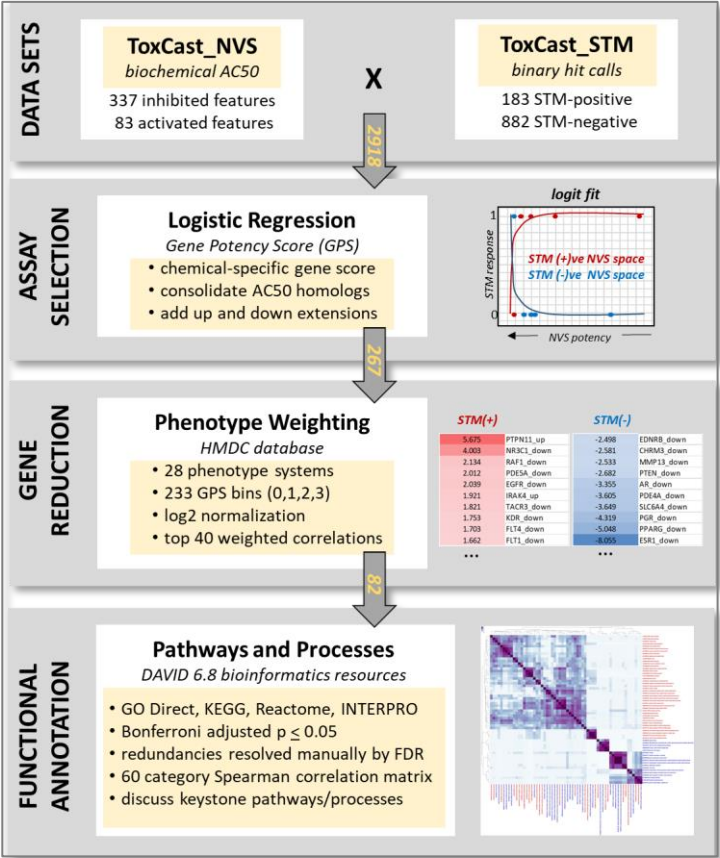


Cyclopamine

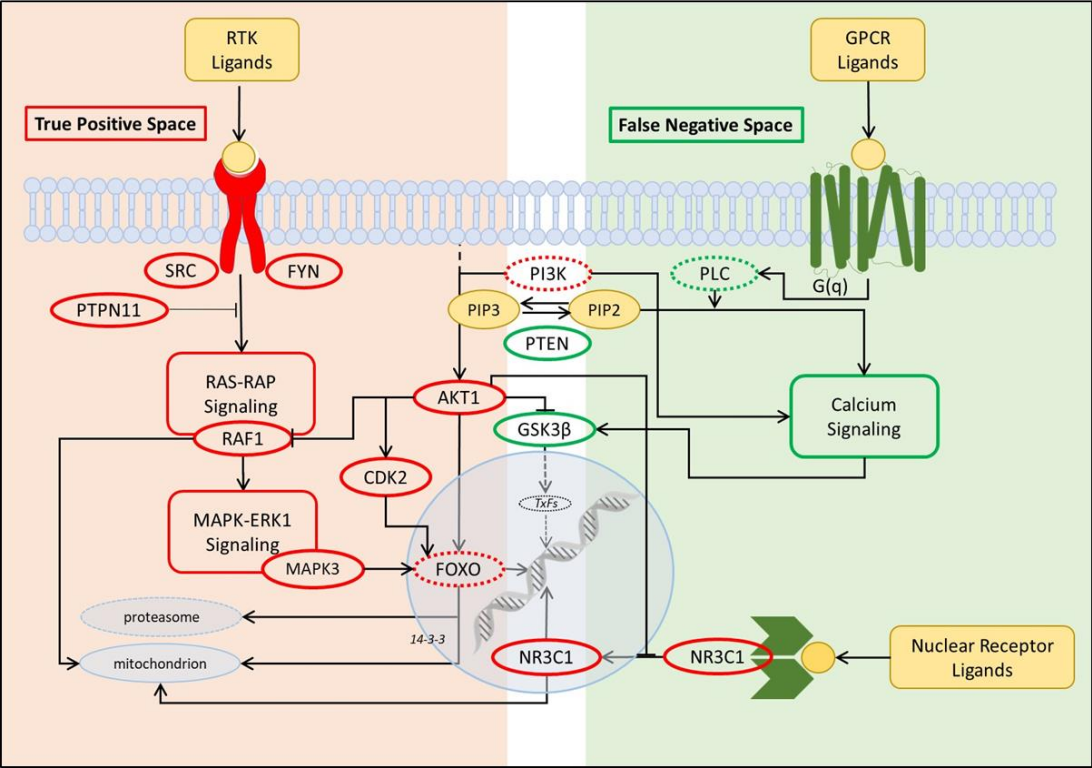
TI = NA, CV = NA

What human relevant pathways are detected or missed?

Workflow to mine hPSC bioactivity against 337 cell-free biochemical assays (ToxCast_NVS)



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



Sensitive Domain

Insensitive Domain

Performance check for hPSC-based classification of DevTox

- Qualification on 42 well-curated reference compounds often used to validate alternative DevTox platforms¹.
- 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 PSC studies have been validated with a limited set of data-rich chemicals, inflating predictive accuracy >80%.

ATRA was most potent across 1065 compounds tested.

CASRN	Chemical	CV (μM)	TI (μM)		
302-79-4	all-trans-Retinoic acid	NA	0.00		
69-74-5	Cytarabine hydrochloride	0.083	0.00		
59-05-2	Methotrexate	0.062	0.00		
147-24-0	Diphenhydramine hydrochloride	3.76	0.58		
50-35-1	Thalidomide	NA	1.2		
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 hydrochloride	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 sodium phosphate	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
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 monohydrate	NA*	NA	D	FN
56-53-1	Diethylstilbestrol	NA	NA	X	FN
107-21-1	Ethylene glycol	NA	NA	NTP	FN
57-30-7	Phenobarbital sodium	NA*	NA	D	FN
81-81-2	Warfarin	NA	NA	X	FN
69-72-7	Salicylic acid	1795	513	C	TN
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

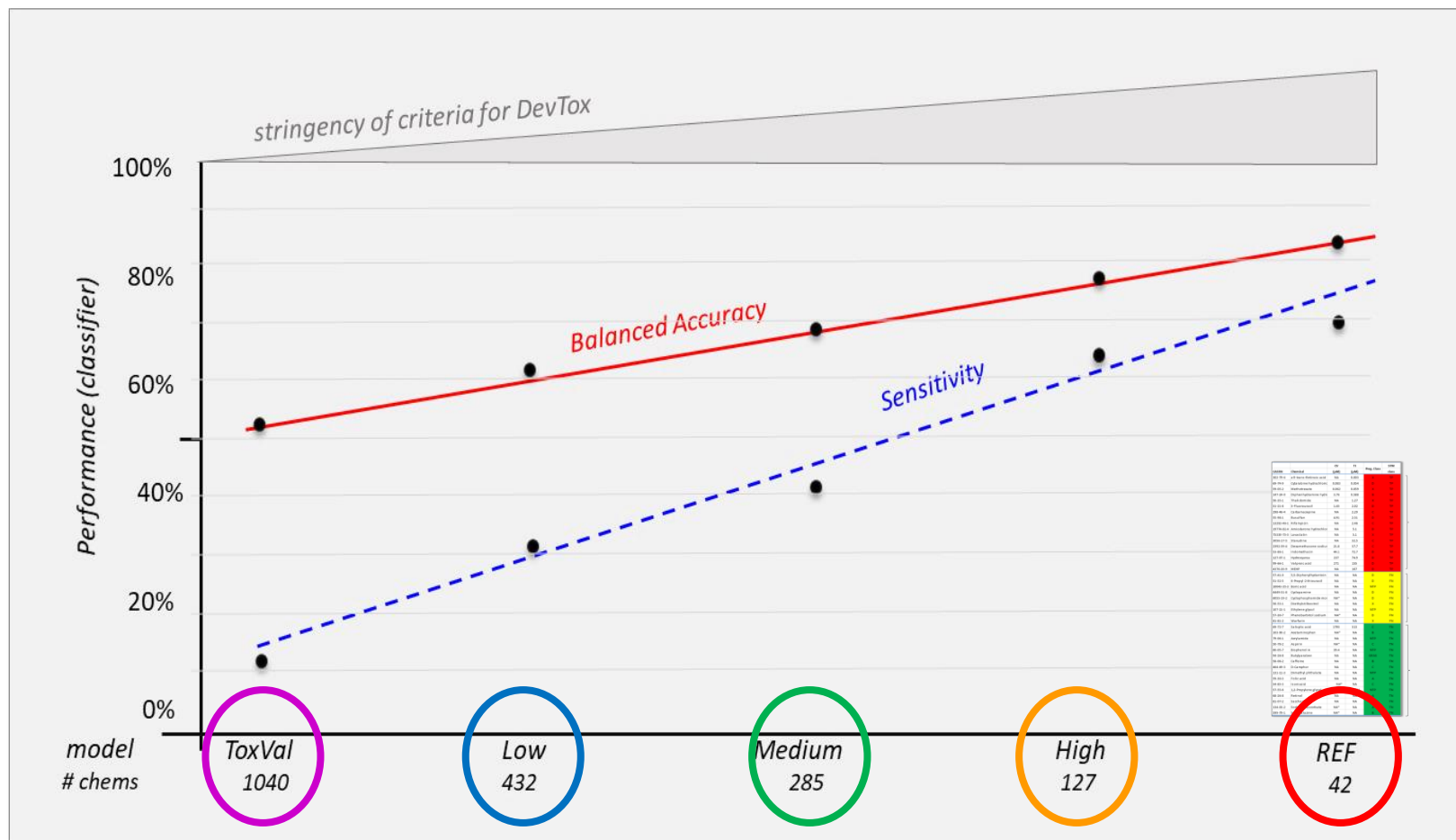
True Positive

False Negative

True Negative

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*)

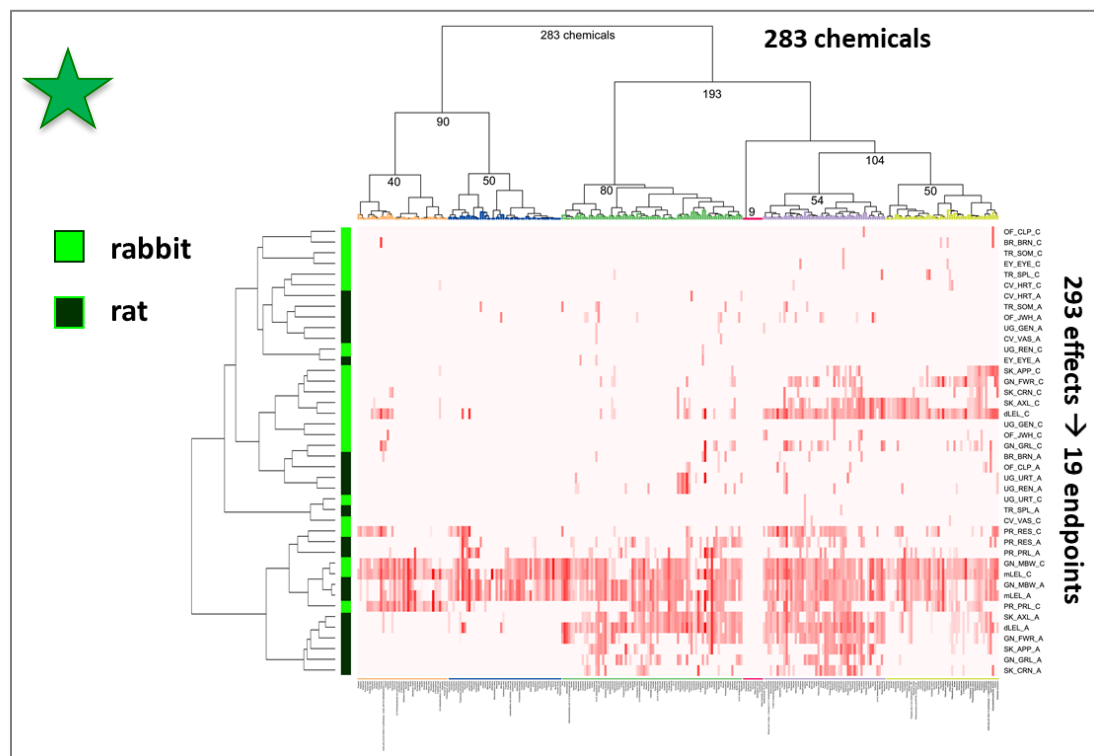


Scaling Criteria (*ToxRefDB*)

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

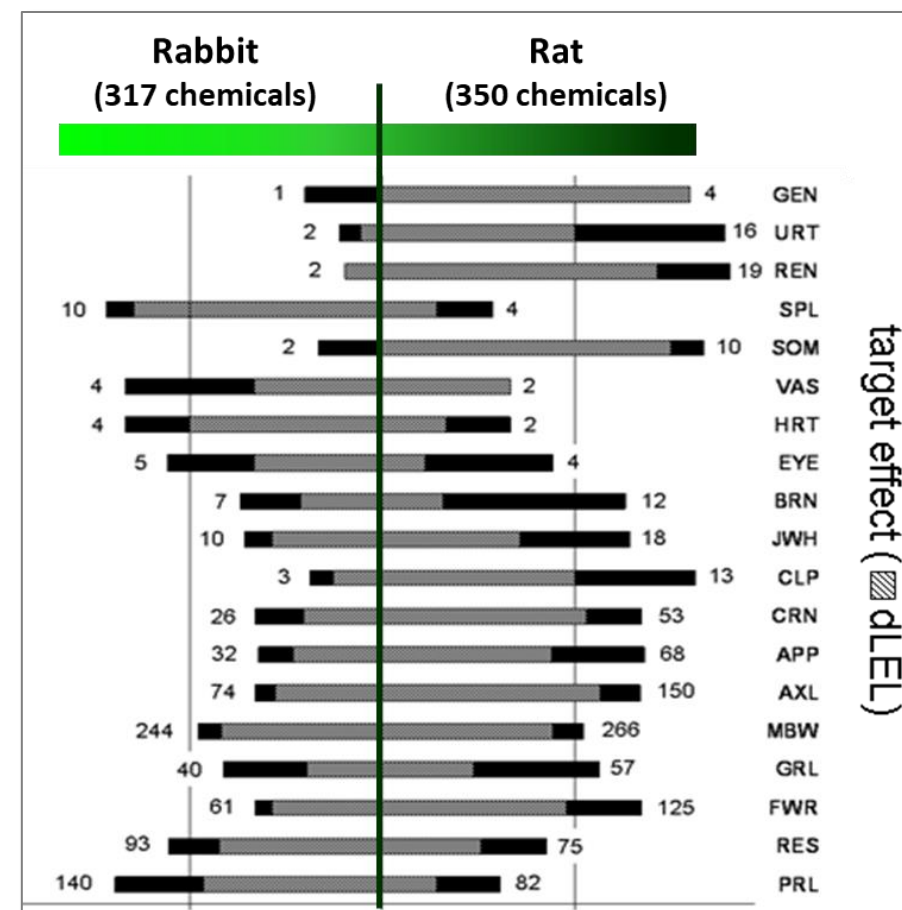
Although *hPSC* positivity rate (19.2%) was similar to concordant rat-rabbit studies (18.7%), only a subset was detected by both platforms.

Prenatal developmental toxicity outcomes in ToxRefDB



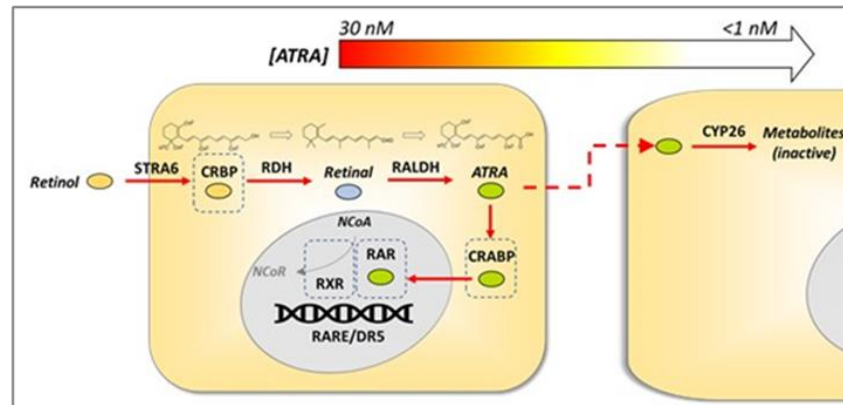
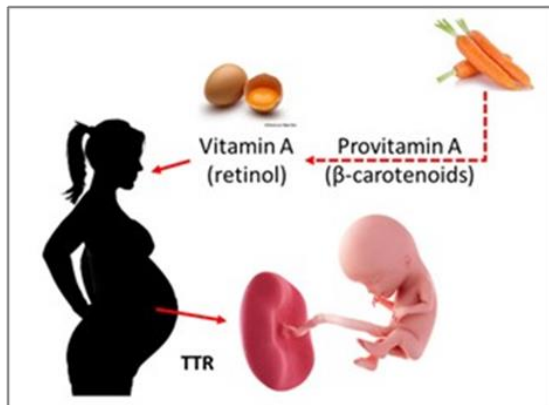
Hierarchical clustering of 283 chemicals (*columns*) by 19 adverse outcomes (*rows*) observed in guideline rat and rabbit studies [*circa 2009*].

Species distribution of adverse outcomes was complex, but the overall pattern is captured by the lowest effect level (dLEL) dose.



Retinoid signaling pathway

The central role of ATRA in patterning and homeostasis of the skeleton makes it a logical choice for case studies in computational toxicology.



- ATRA gradients locally generated by cell-specific expression of enzymes, molecular transporters, and nuclear receptors (RARs) collaborate with powerful morphogenetic signals (e.g., FGF, BMP, SHH, WNT, ...).
- Local regulation of ATRA homeostasis and its disruption may be captured in diverse Adverse Outcome Pathway (AOP) frameworks linking molecular initiating events (MIEs) to developmental phenotypes.

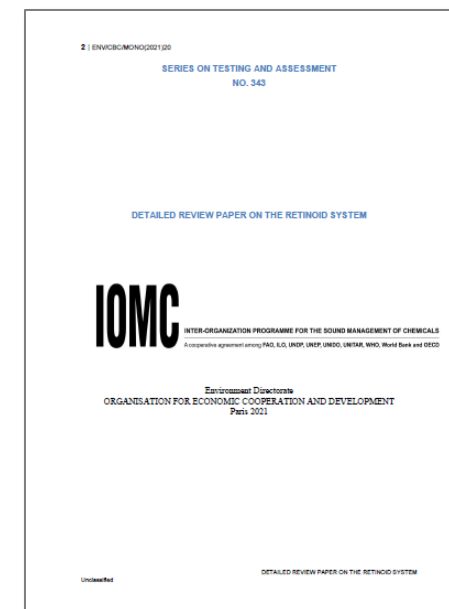
Regulatory interest in the retinoid signaling pathway

2012: DRP 178 of OECD Test Guidelines Programme highlighted a critical need for harmonized regulations on this system for toxicity screening and evaluation.

2015: OECD EDTA Advisory Group initiated DRP Project 4.97 to review the retinoid signaling pathway across diverse organ systems.

2018: DRP 4.97b narrowed to four areas: Overview, Reproductive System (Annex A), Skeletal Patterning (Annex B), and CNS Development (Annex C).

2021: individual publications in *Reproductive Toxicology* (H Hakannson); final report published in *OECD Testing and Assessment Series No. 343* (P Browne).

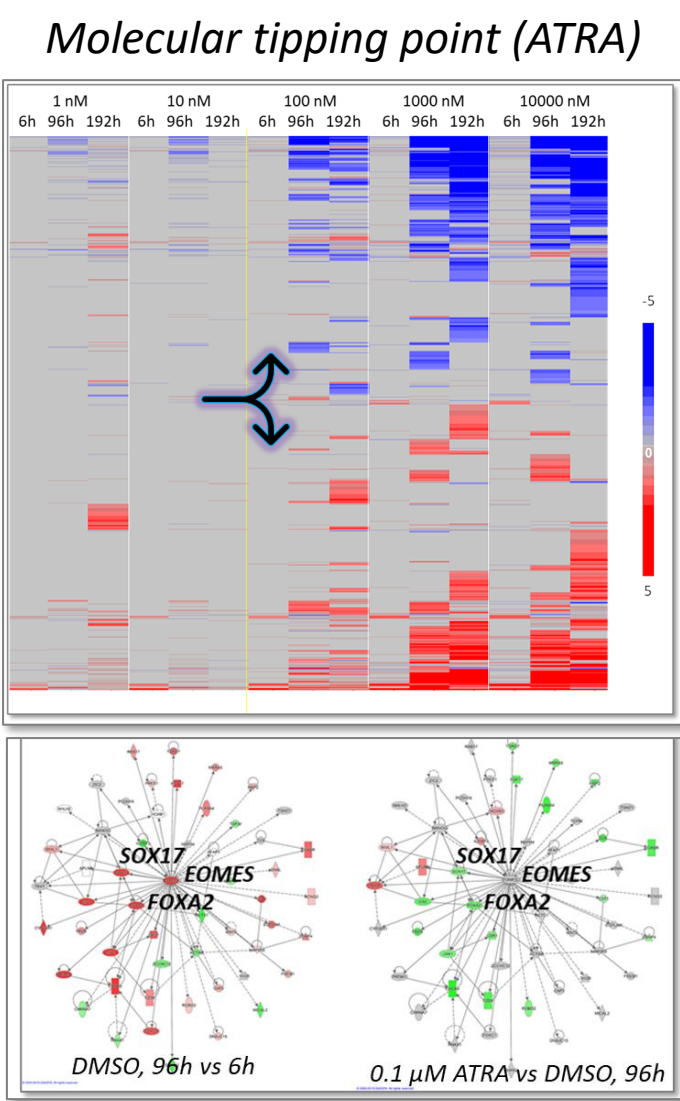


ATRA concentration thresholds and critical response dosimetry

Dosimetric	Conc.	Indication	Reference
baseline ATRA (5 somite zebrafish embryo)	< 1 nM	non-morphogenetic	(Shimozono, Iimura et al. 2013)
maternal serum (animal study)	1.7 nM	non-teratogenic	(Daston, Beyer et al. 2014)
devTOX ^{qp} assay (pluripotent hESC)	3.0 nM	teratogenic threshold	(Zurlinden, Saili et al. 2020)
normal plasma concentration	5.0 nM	physiological (adult)	(Napoli, Posch et al. 1991)
axial gradient (5 somite zebrafish embryo)	6.0 nM	morphogenetic signal	(Shimozono, Iimura et al. 2013)
endodermal differentiation (h-iPSC)	17 nM	toxicological tipping point	(Saili, Antonijevic et al. 2019)
devTOX ^{qp} assay (pluripotent h-iPSC)	19 nM	DevTox potential	(Palmer, Smith et al. 2017)
genetic perturbation (mouse)	30 nM	altered homeostasis	(Helms, Thaller et al. 1994)
maternal serum (animal study)	30 nM	teratogenic potential	(Daston, Beyer et al. 2014)
limb-bud (GD 10.5 mouse embryo)	30 nM	physiological (embryo)	(Horton and Maden 1995)
pharmacological kinetics	1,000 nM	efficacious (therapeutic)	(Helms, Thaller et al. 1994)
limb-bud (GD 11 mouse embryo)	1,500 nM	weakly teratogenic dose	(Satre and Kochhar 1989)
limb-bud (GD 10.5 mouse embryo)	12,500 nM	fully teratogenic dose	(Horton and Maden 1995)

The tipping point between physiological and dysmorphogenic endogenous ATRA is well below fully penetrant teratogenesis.

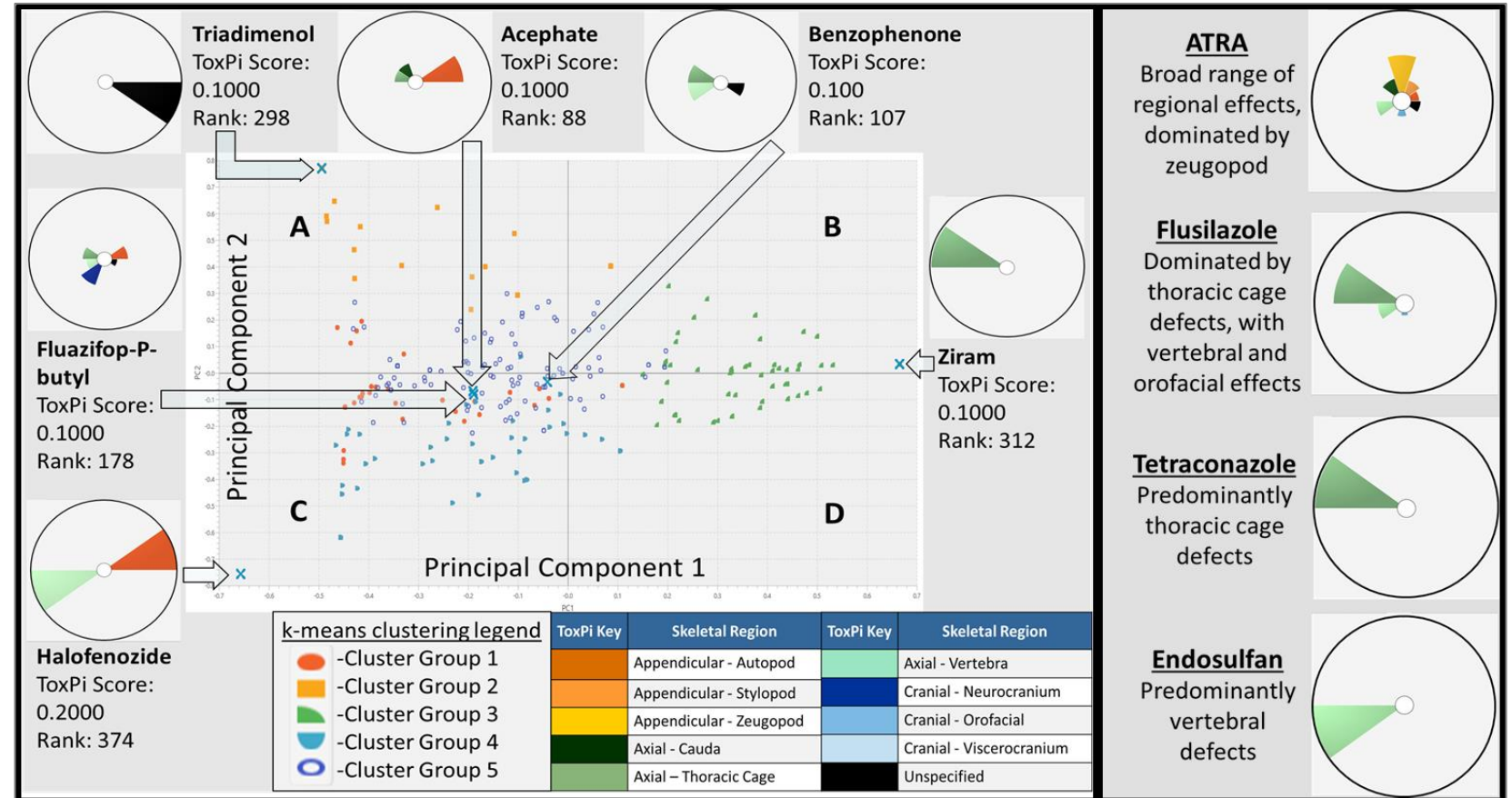
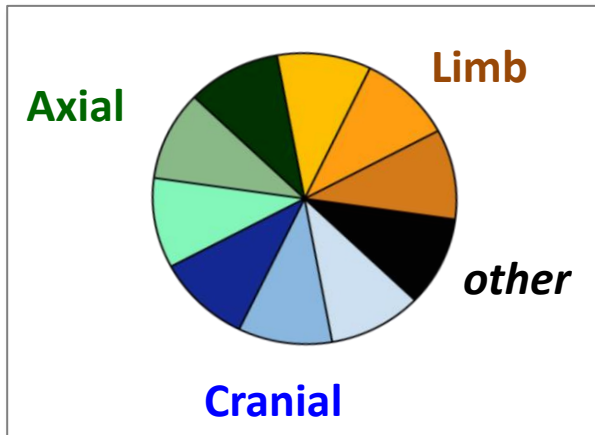
Knudsen et al. (2021), Reprod Toxicol



Saili et al. (2019) Reprod Toxicol

ToxRefDB chemicals (370) clustered by regional phenotype

- Culled fetal effects data from 2,946 ToxRefDB studies;
- 57,198 skeletal defects across rodent/nonrodent studies;
- clustered chemicals (k=5) by phenotypic domains (ToxPi).



Are fetal skeletal phenotypes consistent with AOPs linked to disruption of the ATRA signaling pathway?

Availability of *in vitro* profiling data for the ATRA system

Retinoid Pathway Targets

Retinol Binding Proteins (plasma and cellular transporters)

Molecular transporters for retinol uptake (STRA6, STRA8)

Retinol Dehydrogenase (RDH10)

Retinaldehyde Dehydrogenase (RALDH2)

Cellular Retinoic Acid Binding Proteins (CRABP-I, CRABP-II)

Retinoic Acid Receptors (RARs) alpha, beta, and gamma

Retinoid X Receptors (RXRs) alpha, beta, and gamma

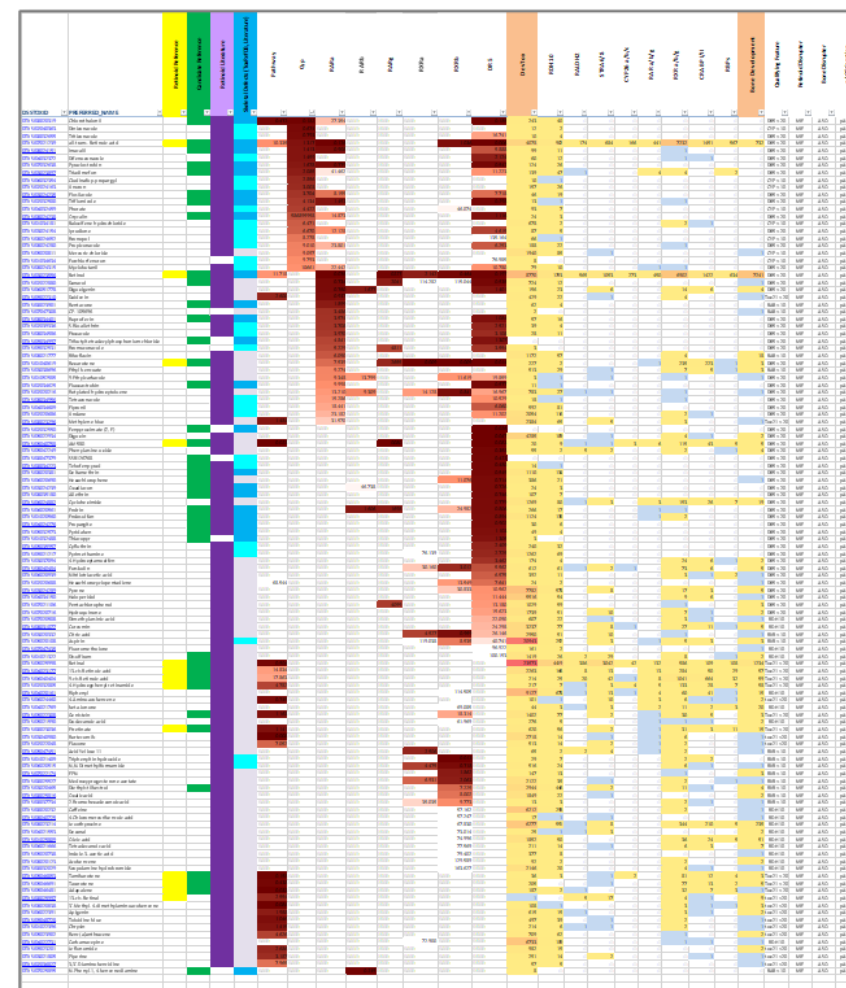
Nuclear Coactivators (NCOAs) and Corepressors (NCORs)

Cytochrome P450 family 26 (CYP26A, CYP26B, CYP26C)

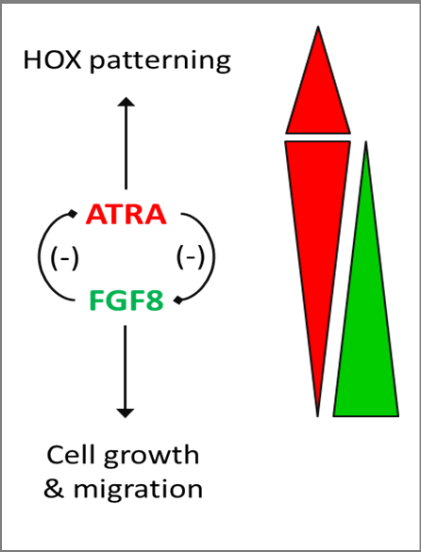
Data available on 117 chemicals to model skeletal embryopathies linked to disruption of ATRA metabolism and signaling.

- ChEMBL data on 12 metabolic assays for drug-like compounds;
- ToxCast HTS data on 11 reporter assays for ~2K chemicals;
- Tox21 HTS data on an intact retinol signaling pathway for ~10K chemicals;
- Potential disruption of ATRA signaling identified for 213 compounds;
- Literature mining (AbstractSifter v5.7) → 5903 related publications.

Chemicals (n=117) In vitro biactivity (k=8 assays) Literature (k=8 assays)



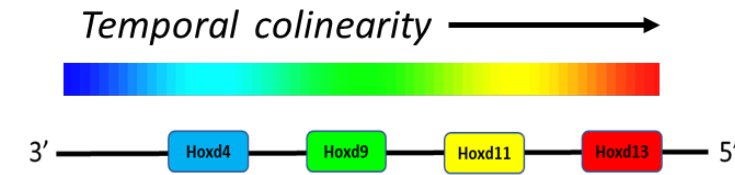
Putative AOPs for ATRA-dependent skeletal embryopathy



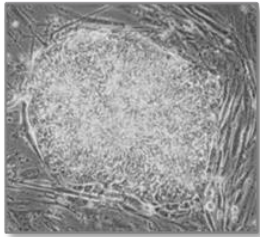
REGION	MIE	KE1	KE2	KE3	KE4	KE5	AO
Anterior Neural Tube	Inhibition of CYP26A1 enzymatic activity	Local increase in endogenous ATRA levels	Hyperactivation of the RAR/RXR heterodimer	Repression of <i>Fgf8</i> limits FGF8 signaling	Mis-specification of CNC cell fate and behavior	Maxillary arch dysplasia alters palatal outgrowth	Cleft palate
Paraxial Mesoderm	Reduction in RDH/RALDH2 activity	Local decrease in endogenous ATRA levels	Hypoactivation of the RAR/RXR heterodimer	Overextension of FGF8 signaling	Disruption of the periodic somitic wavefront	Altered somite number, shape, and alignment	Hemivertebra
Limb-Bud Mesoderm	Hyperactivation of the RAR/RXR heterodimer	Underextension FGF8 signaling from the AER	Dysregulation of <i>Meis1/2</i> and <i>Hox</i> gene expression	Proximalization of the limb-bud mesenchyme	Mis-specification of precartilaginous blastema	Malformed cartilaginous bone rudiment	Phocomelia

Although it is not clear which AOPs may be attributable exclusively to a retinoid-related mechanism, the unifying theme is mutual ATRA and FGF signaling.

Patterning: *computable emergence of regional mesoderm*



Embryoid
Body



Epiblast

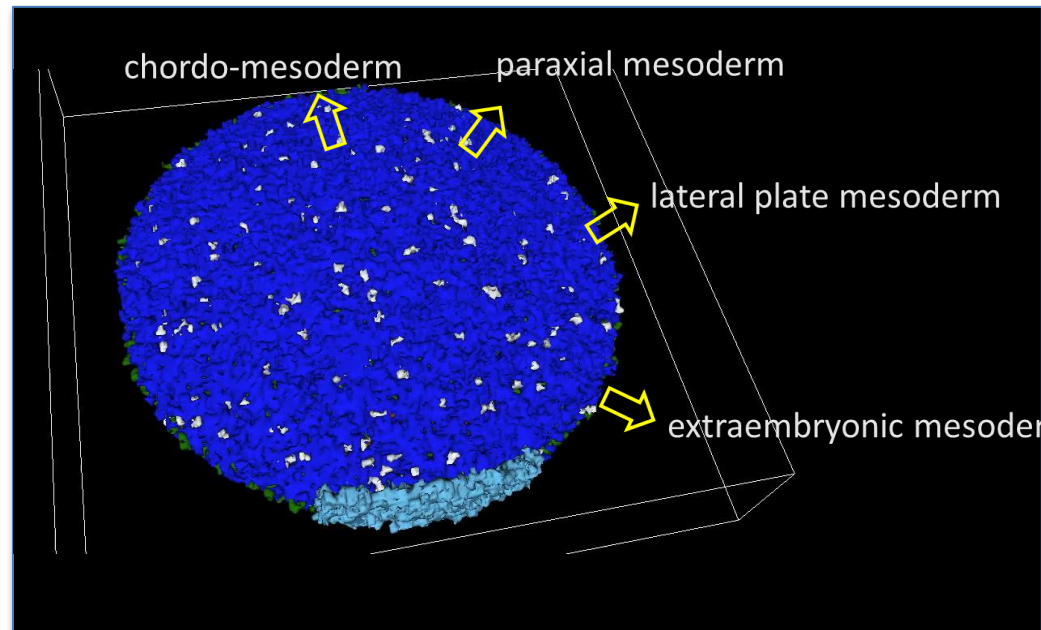


Kyoto Collection

Primitive
Streak



- Cultured hPSCs most closely represent the 'epiblast' of an early embryo during gastrulation (3rd week human), the hallmark of which is the primitive streak (PS).
- Cell migration through the PS is an early determinant of mesodermal fate locking homeobox patterns in '*decoding the genomic blueprint of the fetal body plan*'.



- Input parameters: dynamic signals (eg, FGF2), autonomous HOX clock.
- Stochastic determinants: cell position, timing of migration through PS.
- Emergent property: computable cell numbers for anatomical destiny.
- Editable features: kinematics of signaling, rate of HOX clock.

R Spencer (EMVL) - CompuCell3D.org model

Morphing NAMs data across levels of biological organization



- Vast collections of bioactivity data from *in vitro* chemical profiling are now in hand (<https://comptox.epa.gov/dashboard>).
- These complex datasets provide a new resource to examine key cellular and molecular determinants of developmental toxicity.
- However, virtual reconstitution of a self-organizing system from unidimensional data (embryogeny) remains a challenge.

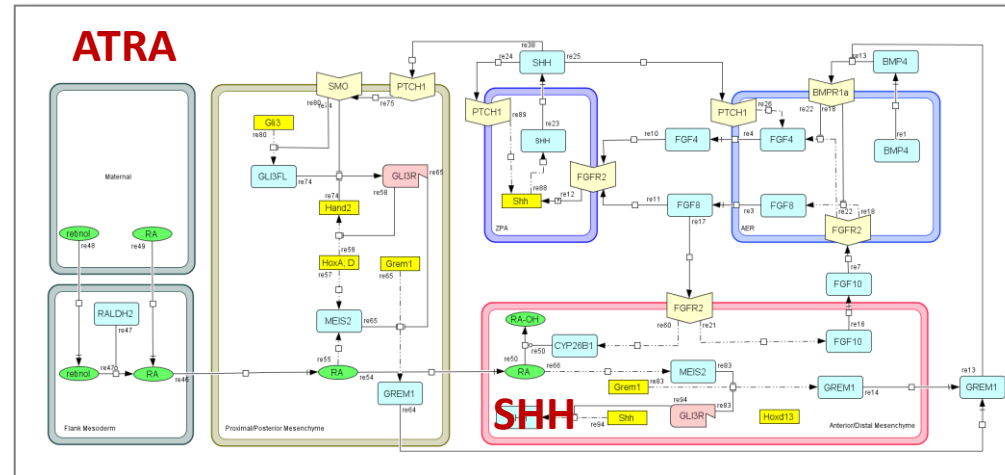
Tactic: build computer models that re-compose human biology and can utilize NAMs data for dynamical simulation and photorealistic rendering.

Cybermorphs: cellular ‘agents’ in a self-organizing system executed computationally into a spatially-perturbed morphology reconstructing effects of an ATRA overload.

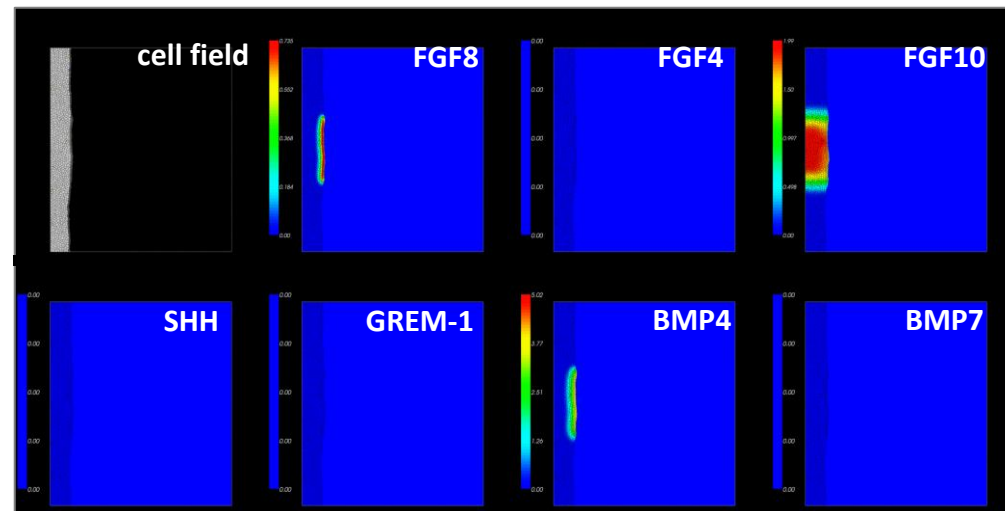


Early limb development
(~4-weeks gestation)

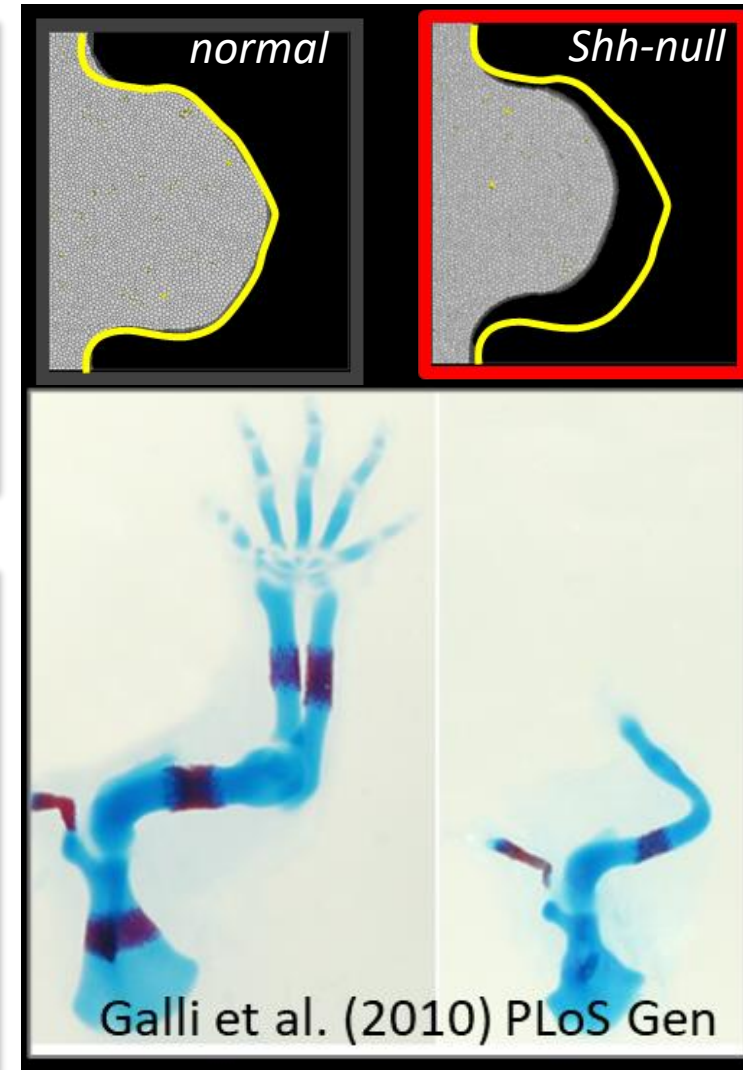
Control system driving polarized outgrowth



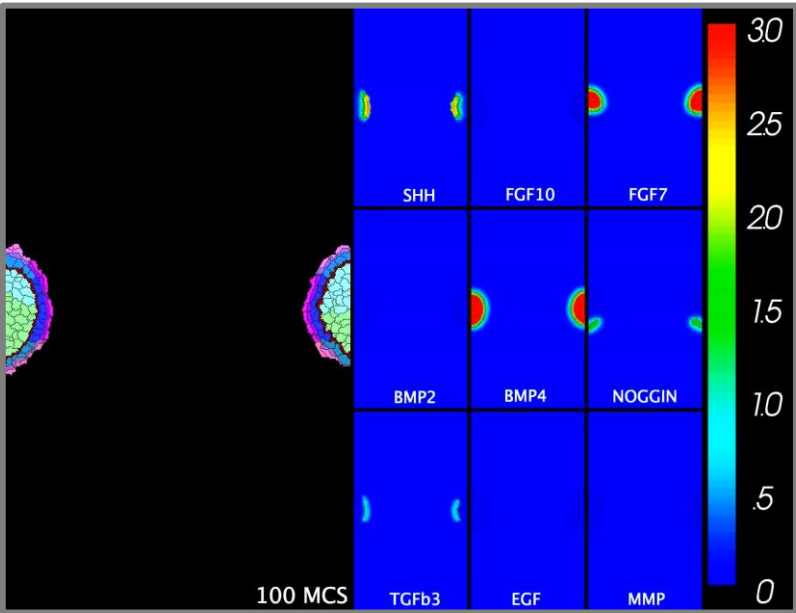
Cell agent-based model (compucell3d.org)



Hacking the biology

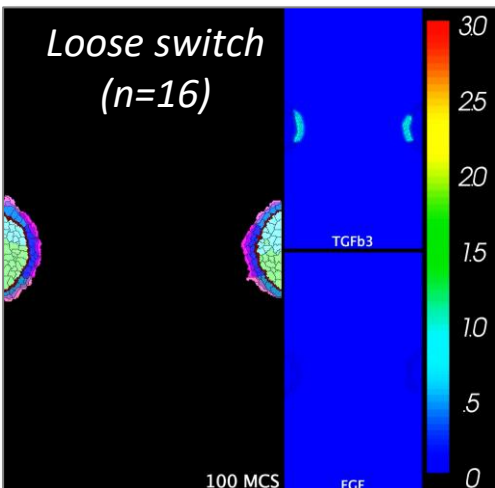
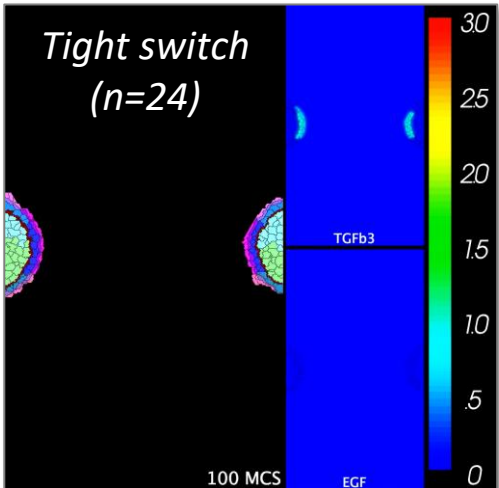
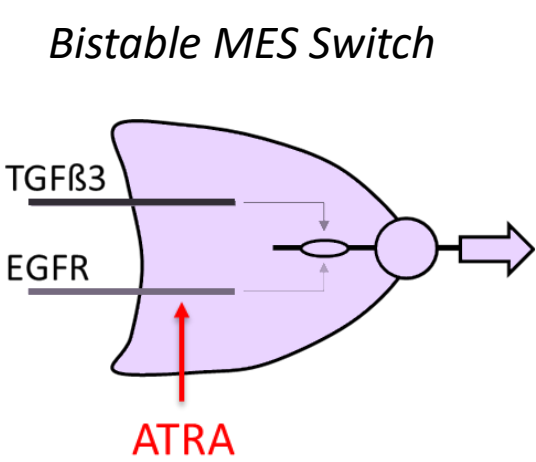
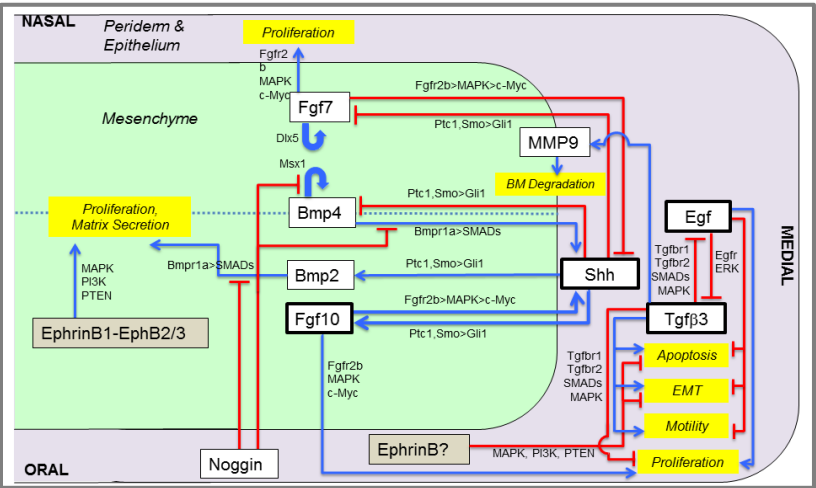
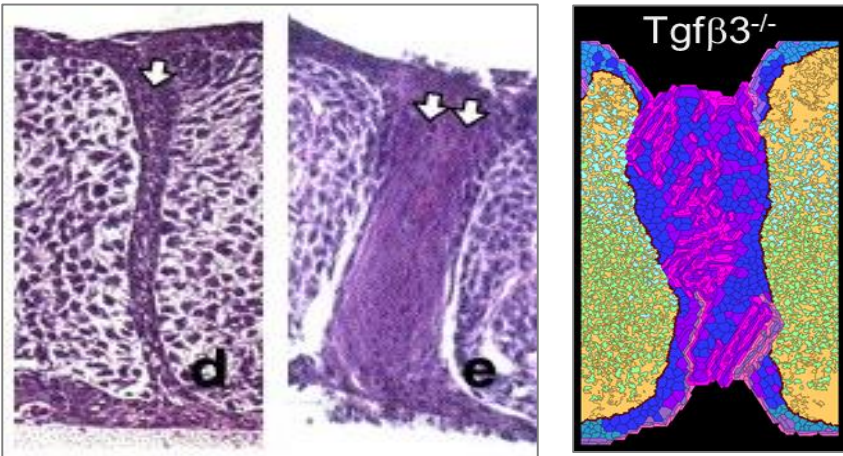


Smart models: specifying the right biology enables real outcomes to self-organize.

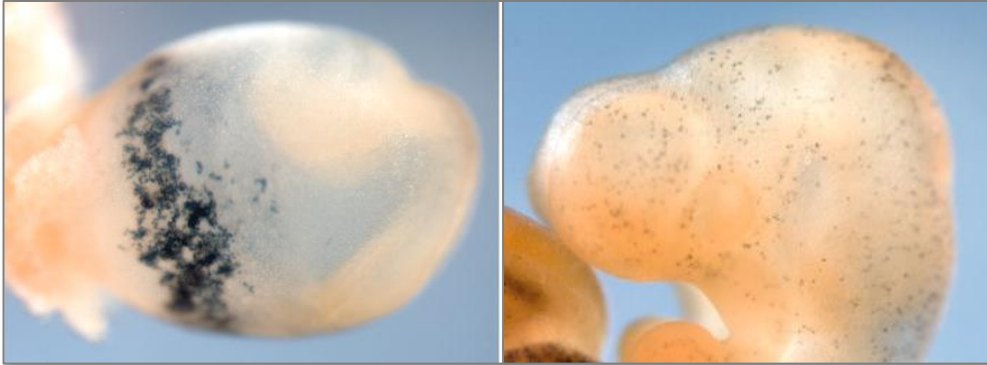


Reviewer Comment: “Crucial mechanisms occurring during palate fusion, especially opposing palatal shelf adhesion, are not considered in the model. ... Even in those strains in which palatal shelves adhere partially, I have never seen a MES as the one shown in Fig. 5.”

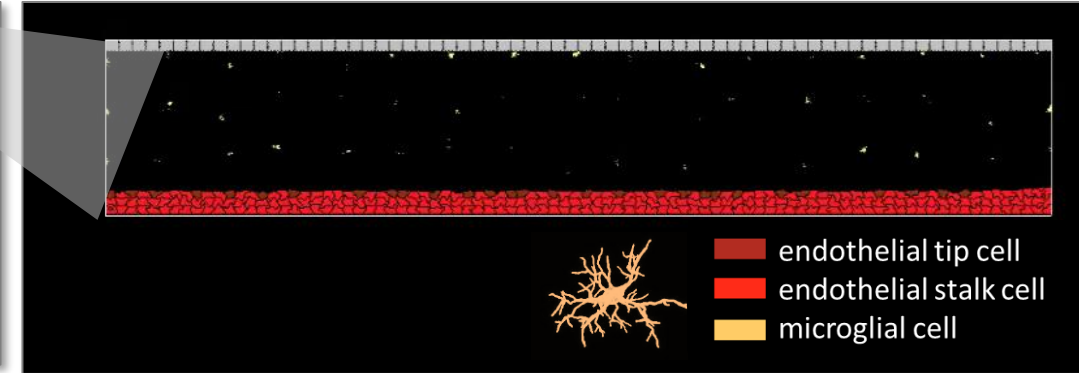
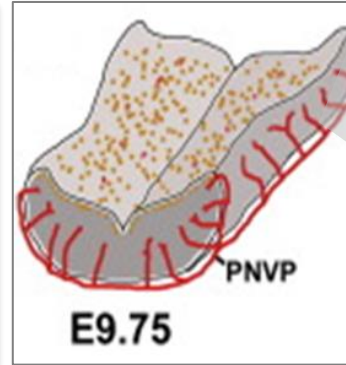
Our Response: TGF-b3 knockout palates *in vitro* (Dudas et al. 2004).



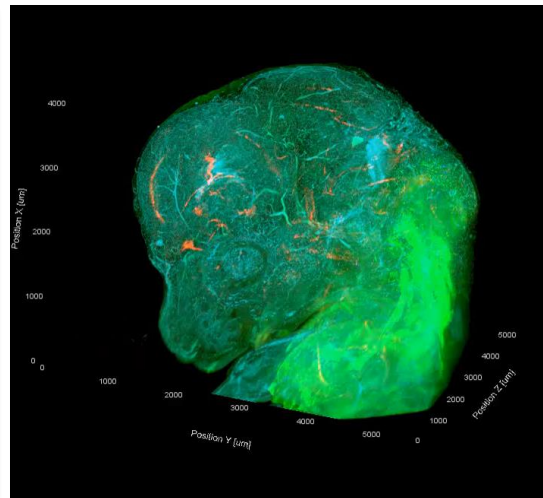
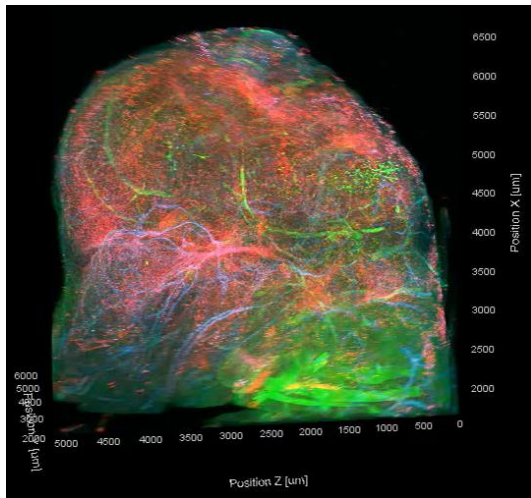
Computational neurovascular unit (cNVU)



Microglia originate from the yolk sac (E8) and pepper the neuroepithelium (E9)
Ginhoux et al. (2010) Science



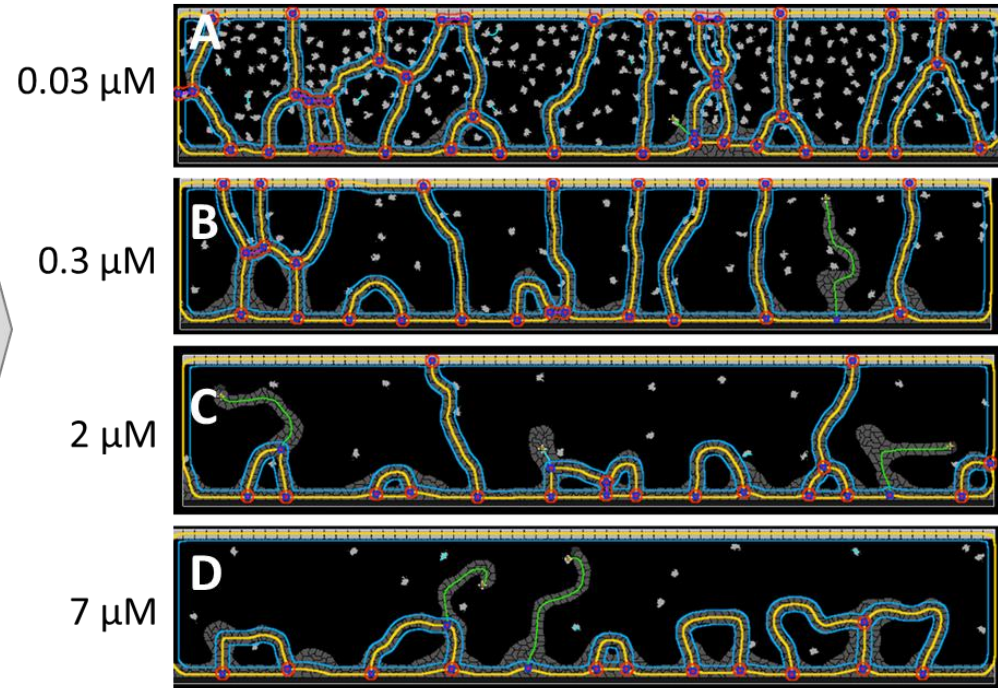
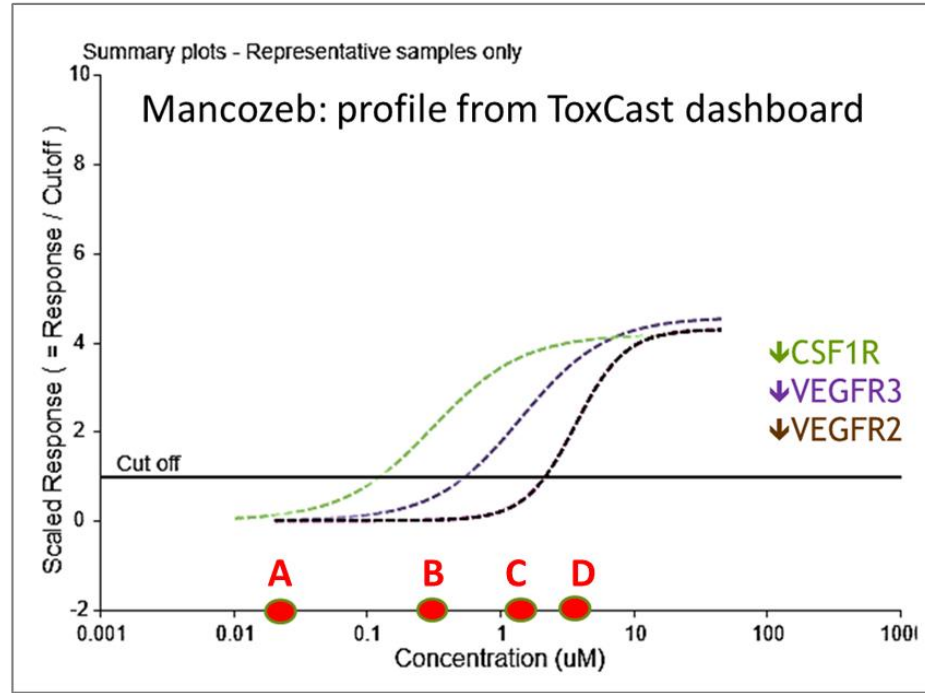
Microglial-endothelial interactions with angiogenic sprouts from PNVP promote BBB microvasculature.



Hypothesis: microglia cells promote microvascular development as sensors of the microenvironment and actuators of neuroinflammatory processes.

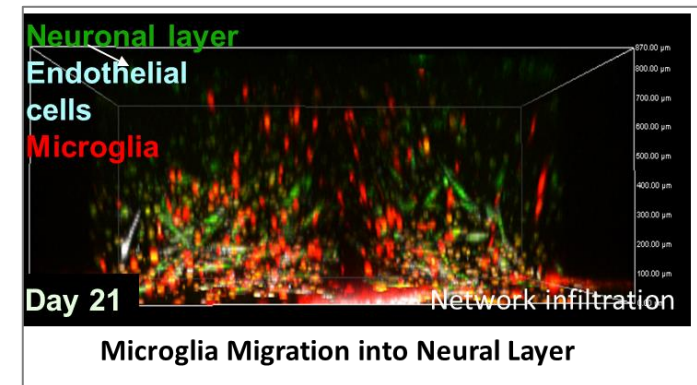
*EPA-A*STAR collaboration with F Ginhoux (work in progress)*

Translation of HTS concentration-response into phenotype



- HTS bioactivity profile for three receptors known to mediate microglial-endothelial interactions (predicted LEL = 0.5 μM).
- Critical effect in an engineered PNVP microsystem for microglial cell migration (observed LEL = 0.3 μM) [Kaushik et al. (2020)].

Compucell3D model (Naphade et al., manuscript in preparation)

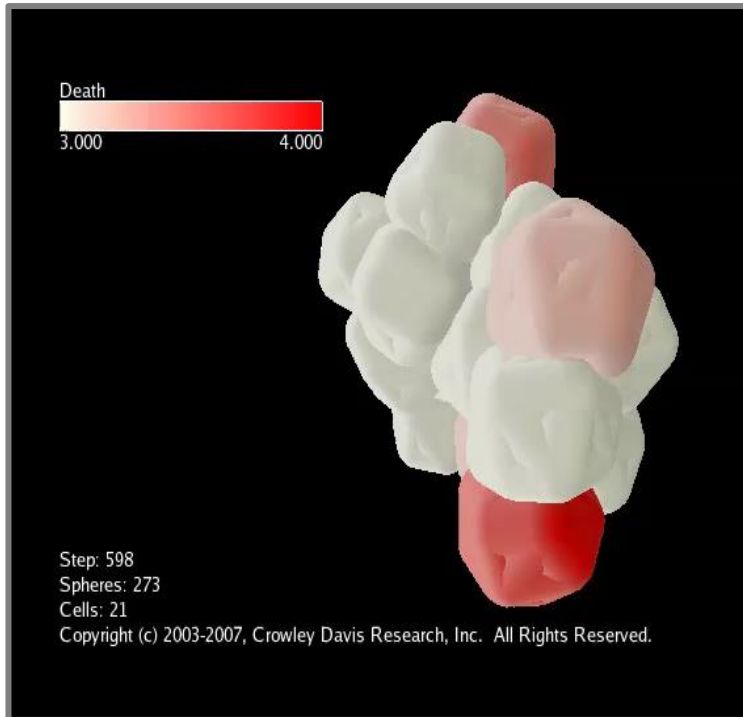


Synthetic Embryology

See the June 2021 issue of 'Development'
devoted to 'synthetic embryology'



*Anatomical homeostasis in a
self-regulating 'Virtual Embryo'*



Andersen et al. (2006) Am. Assoc. A.I.

- **synthetic microsystems:** reconstitute the physical system *in vitro* from hPSCs ('stembryo').
- **computational intelligence:** smart models use fuzzy logic to fill in missing or incomplete information ('synbryo').
- **artificial life:** reconstruct developmental toxicities evolved through automation, control networks ('cybermorphs').

A fully computable synthetic embryo ('synbryo') may be a distant goal, but cinematic representation of time-evolved scientific data is a closer frontier.

Interactive Cinematic Scientific Visualization (iCSV)

Presents scientific data in a way that is understandable and aesthetically pleasing, putting dynamical systems into motion (e.g., award-winning documentaries in planetary science).

Same data displayed by traditional and CSV ... capturing one theory on 'Birth of the Earth'



[video provided by KM Borkiewicz, National Center for Supercomputing Applications (NCAS), Univ. Illinois]

Photorealistic animation can put AOPs into motion in time and space for simple, direct, and impactful translation of complex scientific data to a variety of audiences.

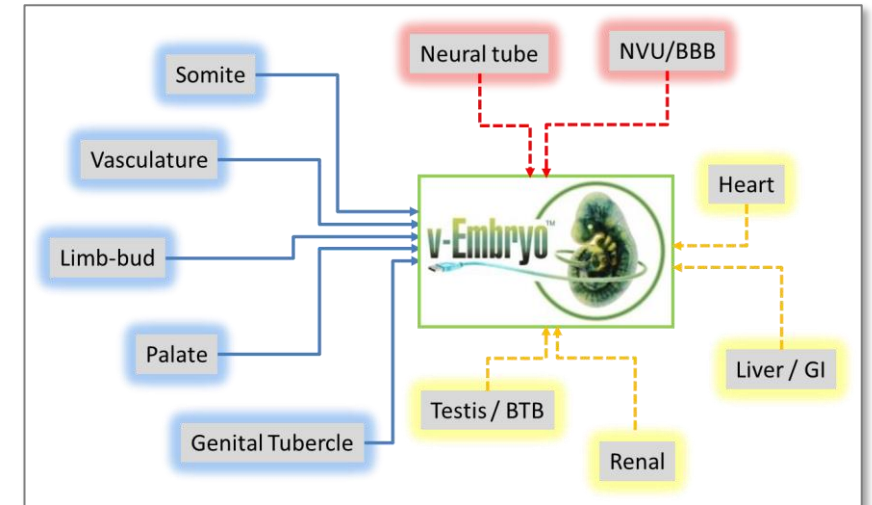
Challenges for predictive toxicology

Computational intelligence: *how complex must the data and models be for synthetic embryology to work?*

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

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

Cinematic visualization: *what new ways can users interact with complex 3D data models in a virtual world?*



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