EPA's Computational Toxicology Community of Practice
October 22, 2020
11:00 am – noon EST

Celebrating Children's Health Month

Researching Developmental Toxicity Models

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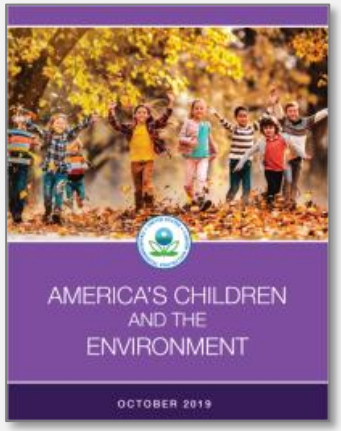
DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

October is Children's Health Month:

Raising Children's Environmental Health Awareness

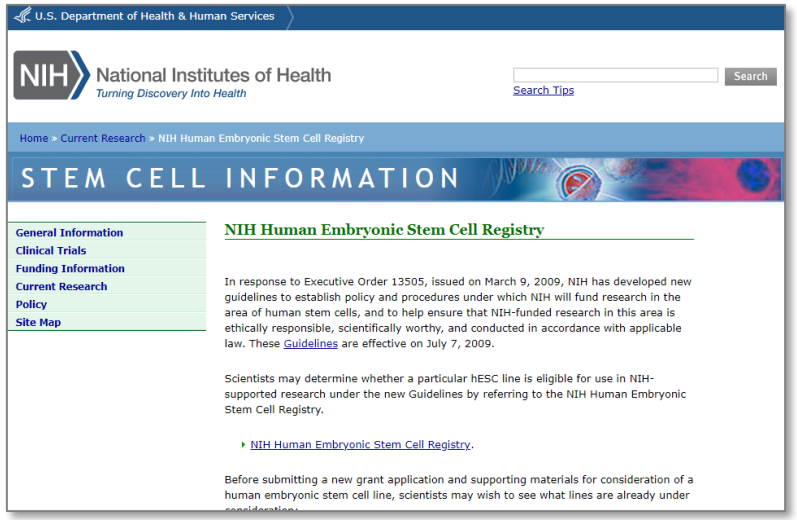


https://www.epa.gov/children/

- Respiratory diseases
- Childhood cancer
- Neurodevelopmental disorders
- Obesity
- Adverse birth outcomes

- Children's health outcomes result from multiple causes:
  - genetics, nutrition, socioeconomic factors
  - prenatal and childhood exposure to environmental factors.
- Adverse birth outcomes:
  - preterm birth, low birth weight, birth defects, infant mortality
  - USA occurrences: ~3% birth defects and 11% low birth weight.
- Assessing developmental toxicity during pregnancy:
  - critical role in setting health and environmental policy
  - teratogenesis is a major concern in animal models of human relevance (e.g., pregnant rats, rabbits).
- New Approach Methodologies (NAMs):
  - shifting developmental hazard detection to *in vitro* data, *in silico* models, and biological pathways
  - embryonic stem cell (ESC) lines are among the most promising alternatives to pregnant animal testing.

Disclosure: *research on pluripotent stem cell lines*



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

Compliance*:* All work involving human embryonic stem (hES) cell lines is compliant with Executive Order 13505 (issued 2009) to ensure that is ethically responsible, scientifically worthy, and conducted in accordance with applicable law.

Funding: This research was performed under EPA's *Chemical Safety for Sustainability Research Program, Research Area 5 'Virtual Tissue Models' (VTMs).*

Our hESC cell lines are registered in the NIH Human Embryonic Stem Cell Registry:

WA09 (H9): NIH Approval Number: NIHhESC-10-0062

RUES2: NIH Approval Number: NIHhESC-09-0013

→ Stemina BIOMARKER DISCOVERY

Stemina Biomarker Discovery
EPA contract EP-D-13-055

Other pluripotent stem cell lines:

Episomal hiPSC Line (Gibco-A18945)

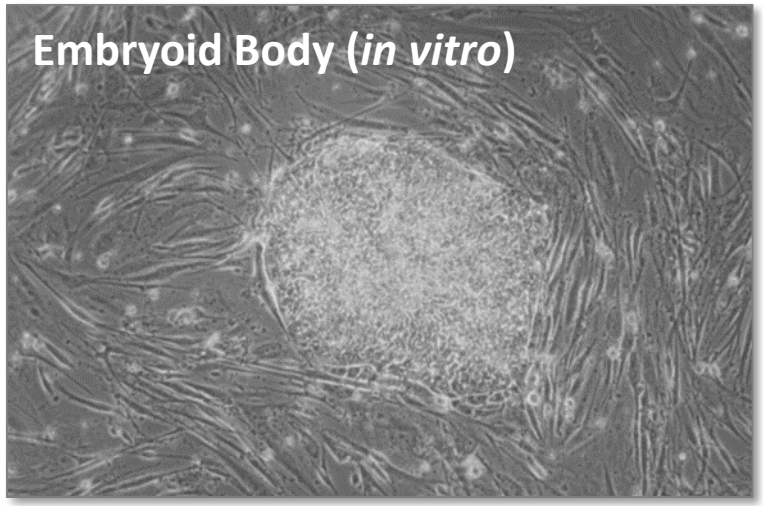
Endodermal hiPSC line (Allele Biotech #ABPSC-HDFAIPS)

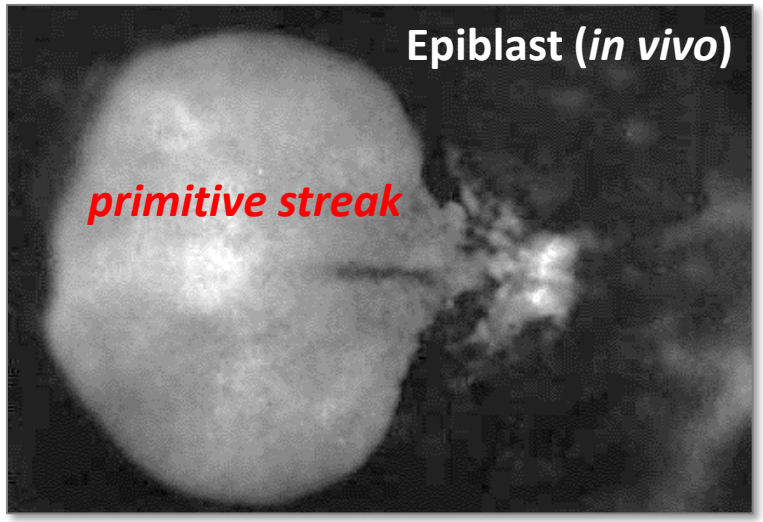
J1 mouse ESC line (ATCC® SCRC-1010™)

→ VALA SCIENCES

Vala Sciences, Inc.
EPA contract EP-D-13-054

Novel features of pluripotent hESC lines:



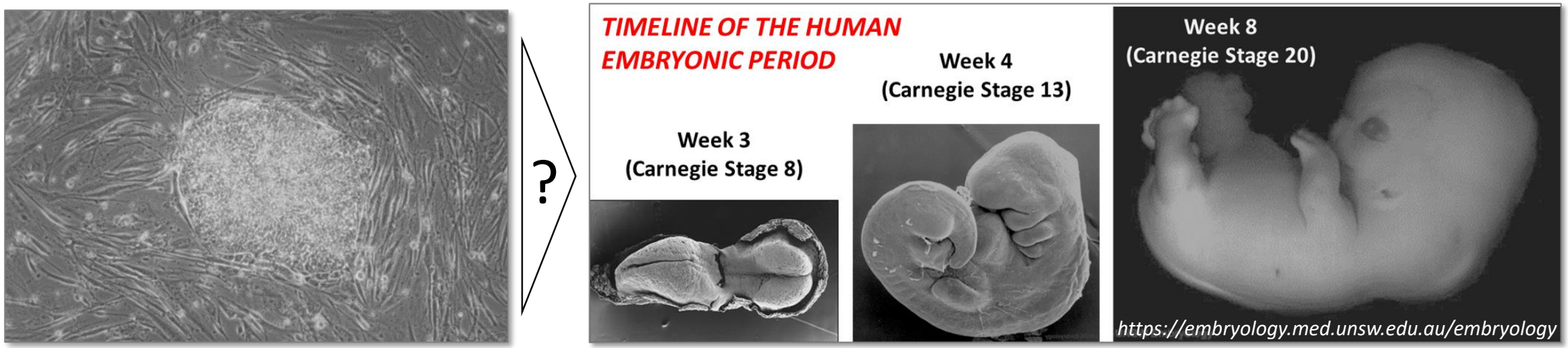


From the Kyoto Collection

- **Self-renewal:** cells proliferate and replicate themselves indefinitely when cultured under appropriate conditions.
- **Differentiation:** maintain the potential to:
  - i) form most cells of the embryo/fetus (pluripotency)
  - ii) self-organize into rudimentary structures (autopoiesis).
- **Embryology:** hESC monolayers recapitulate the 'epiblast' of an early embryo (GD 5-7 mouse, week 2-3 human).

Pluripotent ESC lines capture many key features of embryogenesis that are uniquely covered in guideline prenatal developmental toxicity studies.
(e.g., OECD TG 414, organogenesis in pregnant rat/rabbit)

Will a primitive hESC type live up to the challenge of NAMs?

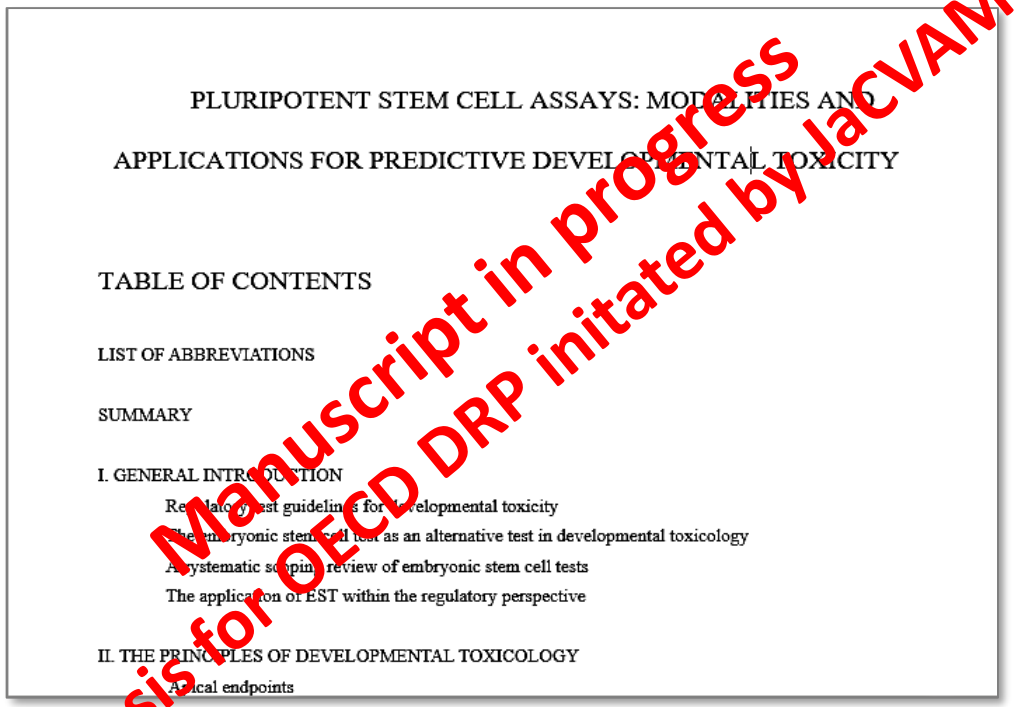


- does not encompass the full complexity of anatomical development;
- blind to the precise spatial-temporal control of cell-cell interactions *in vivo* ;
- misses developmental effects secondary to maternal or placental toxicity;
- uncertainty of post-organogenesis vulnerability and post-natal manifestations;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

A lot of work has been done using mESC and hESC lines *in vitro*

Detailed literature review of conceptual and practical considerations that must be given for readiness of mESC and hESC data in regulatory toxicology (e.g, NAMs).

- chemical domain
- readout endpoints
- standardized protocols
- reproducibility
- biological domain
- predictive power


PLURIPOTENT STEM CELL ASSAYS: MODALITIES AND

APPLICATIONS FOR PREDICTIVE DEVELOPMENTAL TOXICITY

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

SUMMARY

I. GENERAL INTRODUCTION

Regulatory test guidelines for developmental toxicity

The embryonic stem cell test as an alternative test in developmental toxicology

A systematic scoping review of embryonic stem cell tests

The application of EST within the regulatory perspective

II. THE PRINCIPLES OF DEVELOPMENTAL TOXICOLOGY

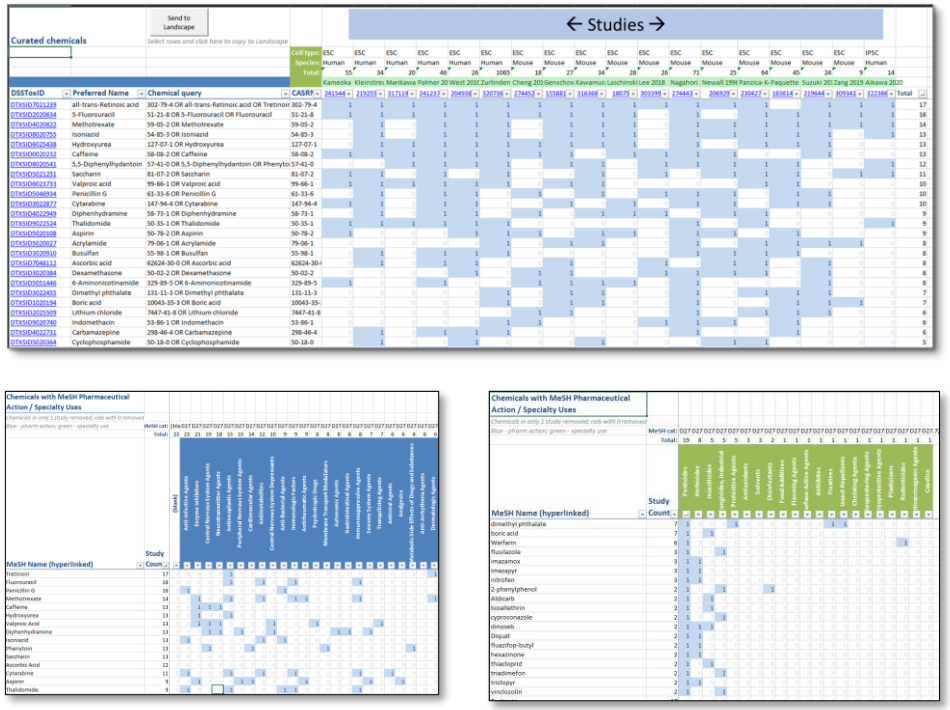
Apical endpoints

Manuscript in progress
(basis for OECD DRP initated by JaCVAM)

N Baker, G Daston, B Flick, M Fujiwara, T Knudsen, H Kojima, A Piersma, H Spielmann, N Suzuki, K Tsaioun (2020)

ESC testing data available in the open literature

1,533 PubMed → 333 (AI relevance) → curation



N Baker, K Tsaioun: Abstract Sifter, SWIFT, MeSH terms, Chemicals Dashboard, … working in unison

- **1,250 annotated chemicals:**
  18 publications tested ≥ 10 compounds (primary)
  174 publications tested 1-9 (evidentiary support)
- **Most overlapping compounds:**
  all-trans retinoic acid (17), 5-fluorouracil (16), and methotrexate (14) - all strong teratogens.
- **Most common use categories:**
  pharma: anti-infective agents, enzyme inhibitors
  chemical: pesticides.

Now includes ToxCast_STM dataset

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 human embryonic stem cell metabolomics assay that "… identified the potential developmental toxicants in the test set with 77% accuracy (57% sensitivity, 100% specificity)."

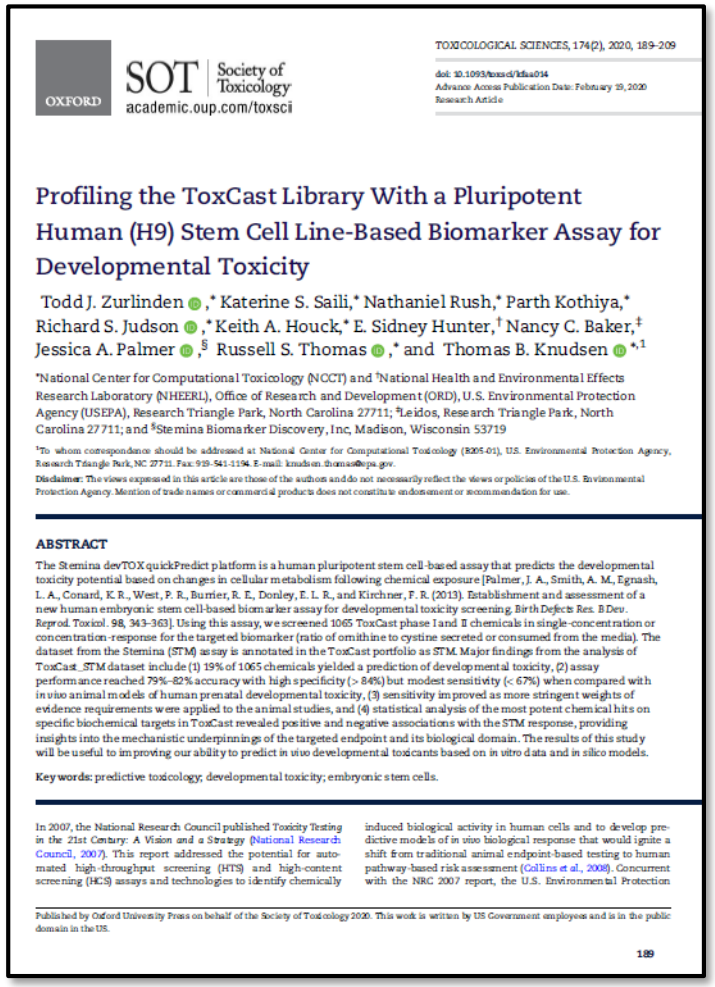
Palmer et al. (2013) Birth Defects Res

Ornithine release
urea cycle, polyamine & pyrimidine synthesis.

TI = ORN/CYSS

Cystine utilization
glutathione synthesis, redox cycling.

Using the H9 devTOX^{qP} platform to profile the ToxCast library



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

TOXICOLOGICAL SCIENCES, 174(2), 2020, 189–209

doi: 10.1093/toxsci/kfaa014
Advance Access Publication Date: February 19, 2020
Research Article

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

Todd J. Zurlinden,* Katerine S. Saili,* Nathaniel Rush,* Parth Kothiya,* Richard S. Judson,* Keith A. Houck,* E. Sidney Hunter,† Nancy C. Baker,‡ Jessica A. Palmer,§ Russell S. Thomas,* and Thomas B. Knudsen*,1

*National Center for Computational Toxicology (NCCT) and †National Health and Environmental Effects Research Laboratory (NHEERL), Office of Research and Development (ORD), U.S. Environmental Protection Agency (USEPA), Research Triangle Park, North Carolina 27711; ‡Leidos, Research Triangle Park, North Carolina 27711; and §Stemina Biomarker Discovery, Inc, Madison, Wisconsin 53719

1To whom correspondence should be addressed at National Center for Computational Toxicology (B205-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711. Fax: 919-541-1194. E-mail: knudsen.thomas@epa.gov.

Disclaimer: The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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, E. L. R., and Kirchner, F. R. (2013). Establishment and assessment of a new human embryonic stem cell-based biomarker assay for developmental toxicity screening. Birth Defects Res. B Dev. Reprod. Toxicol. 98, 343–363]. 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.

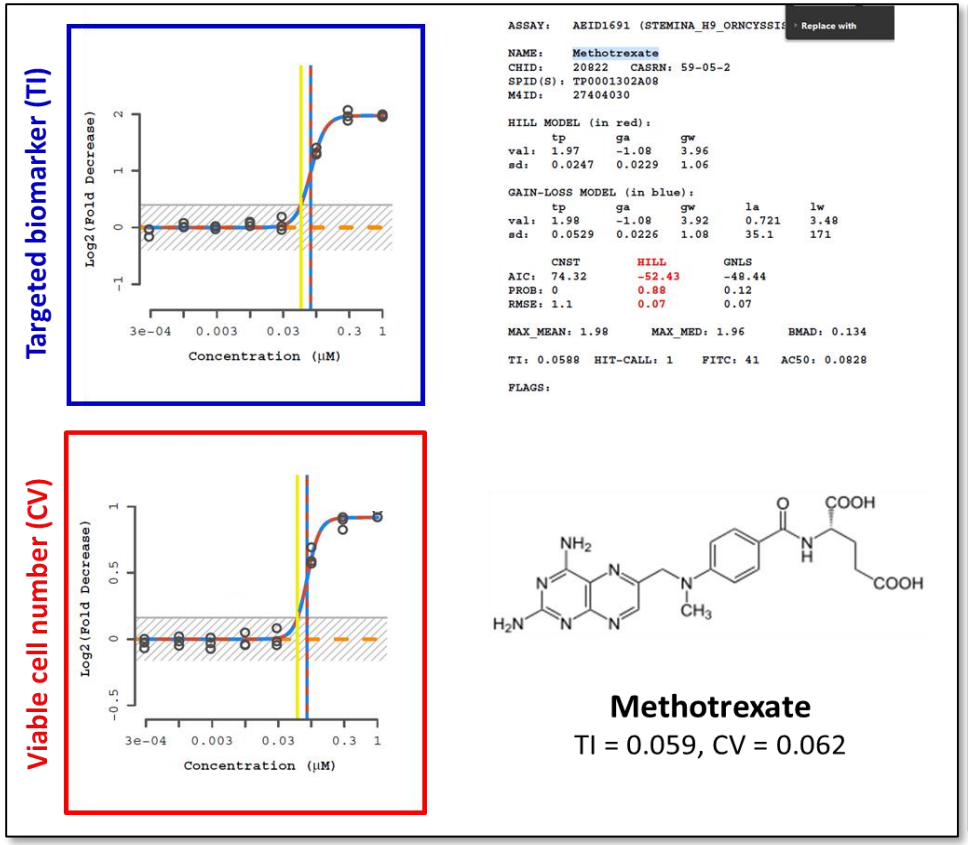
Key words: predictive toxicology; developmental toxicity; embryonic stem cells.

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.

189

Zurlinden et al. (2020) Toxicol Sci



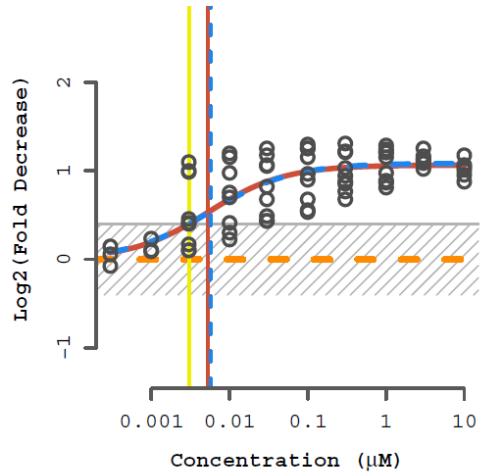
- 1065 ToxCast Ph I/II chemicals at single-conc. or multi-conc.;
- data pipelined to *in vitro-db_v3* database (>1125 features);
- available in EPA's CompTox Chemicals Dashboard;
- ***ToxCast_STM*** dataset includes controls for data quality.

https://comptox.epa.gov/dashboard

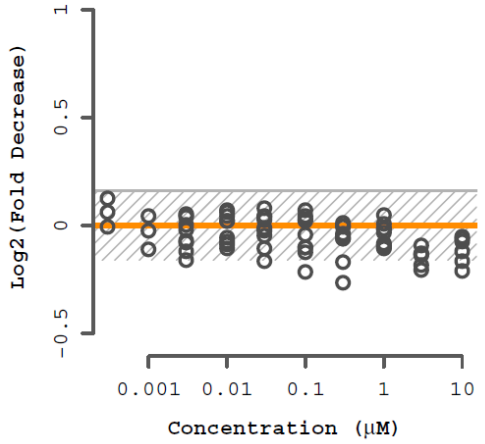
19% positivity rate, indicative of teratogenic potential as a potential developmental hazard prediction.

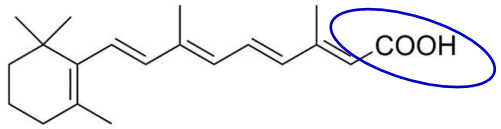
Example: *vitamin-A and its morphogenetic metabolite (all-trans Retinoic acid)*

Targeted biomarker (TI)



Viable cell number (CV)



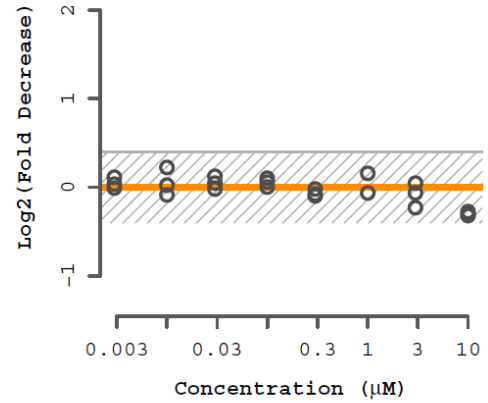


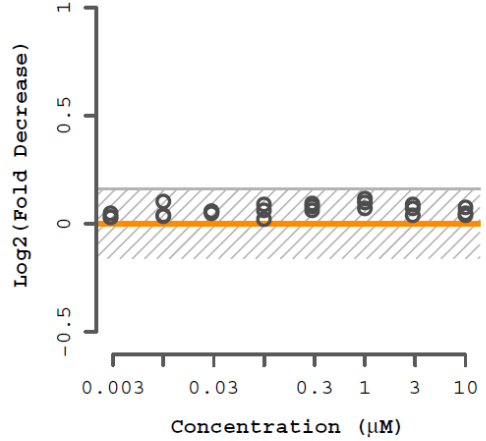
all trans Retinoic acid

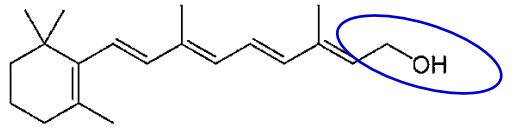
TI = 0.003 µM, CV = NA

dLEL rat = 2.5 mg/kg/day (> mLEL)

dLEL rabbit = 0.5 mg/kg/day (= mLEL)







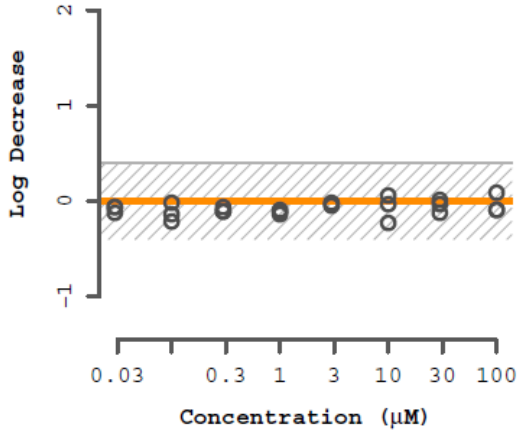
Retinol (vitamin-A)

TI = NA, CV = NA

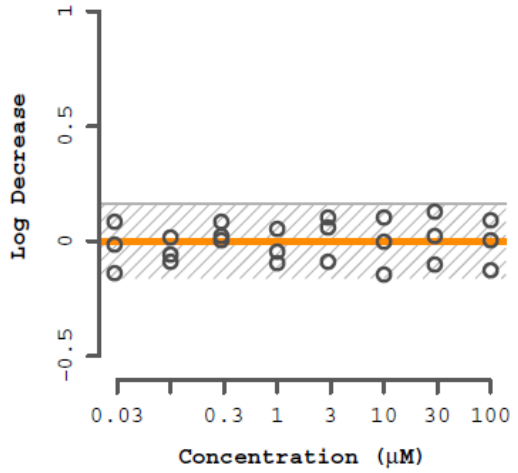
(True Negative)

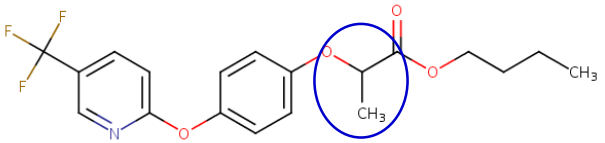
Example: *R-enantiomer (Fluazifop-P-butyl) is the active herbicide*

Targeted biomarker (TI)



Viable cell number (CV)



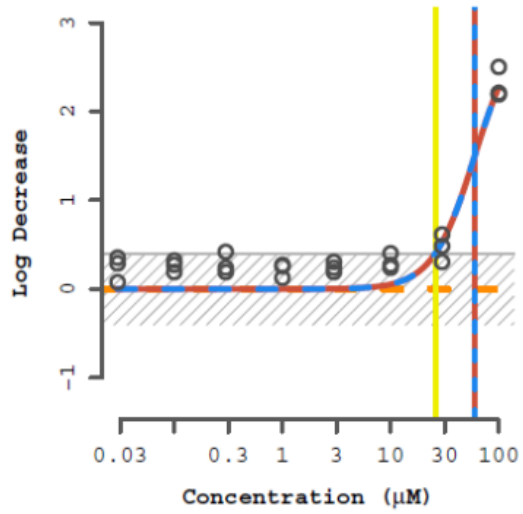


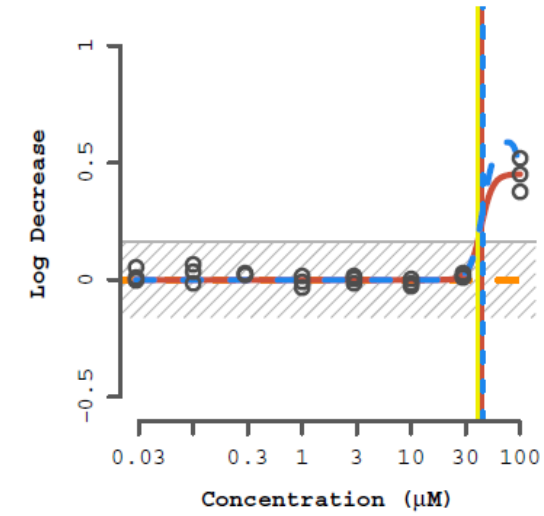
Fluazifop butyl

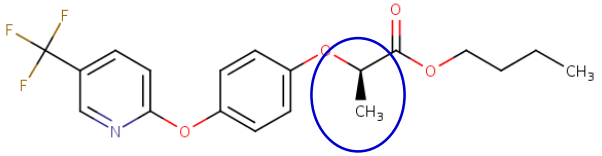
TI = not active, CV = no effect

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

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







Fluazifop-P-butyl

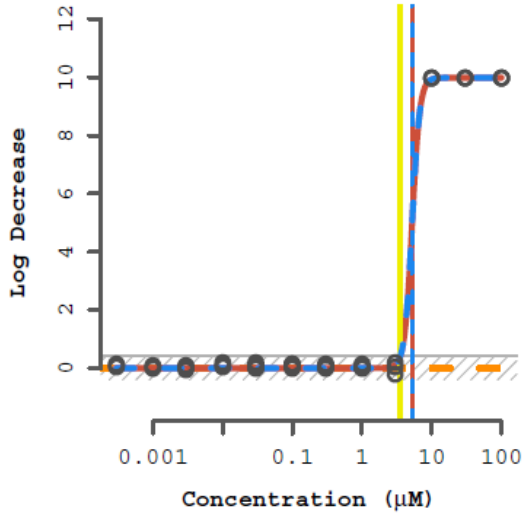
TI = 26 µM, CV = 40.8 µM

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

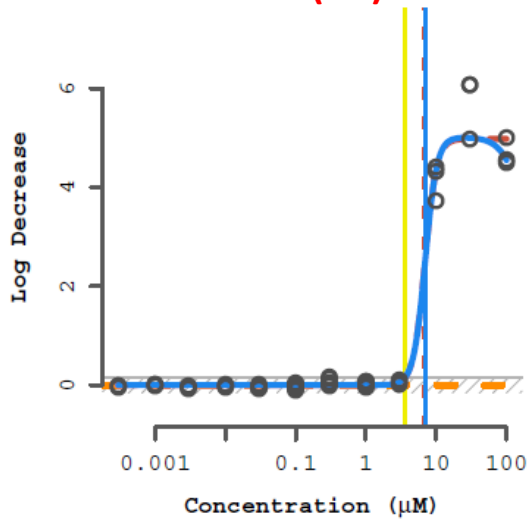
dLEL rabbit = 50 mg/kg/day (mLEL)

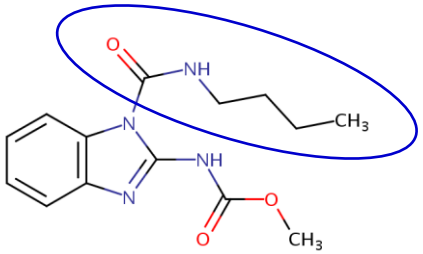
Example: *Benomyl and its conversion product (Carbendazim)*

Targeted biomarker (TI)



Viable cell number (CV)



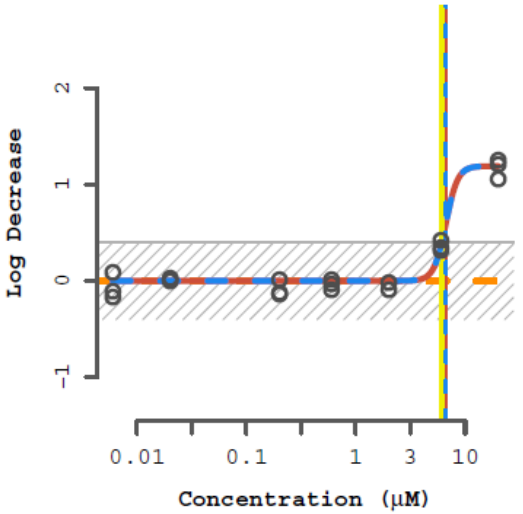


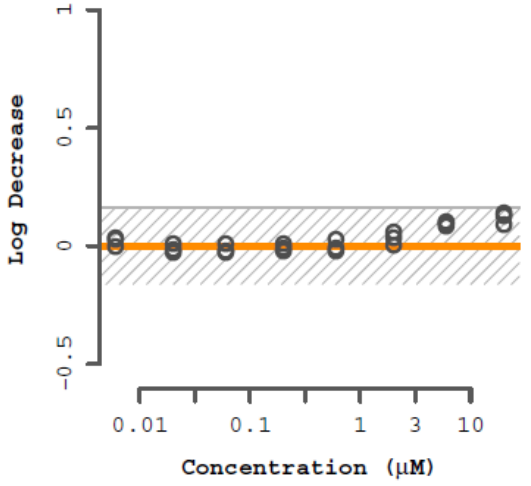
Benomyl

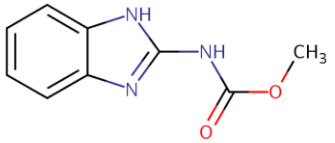
TI = 3.53 µM, CV = 3.63 µM

dLEL rat = 62.5 mg/kg/day (< mLEL)

dLEL rabbit = 180 mg/kg/day (mLEL)







Carbendazim

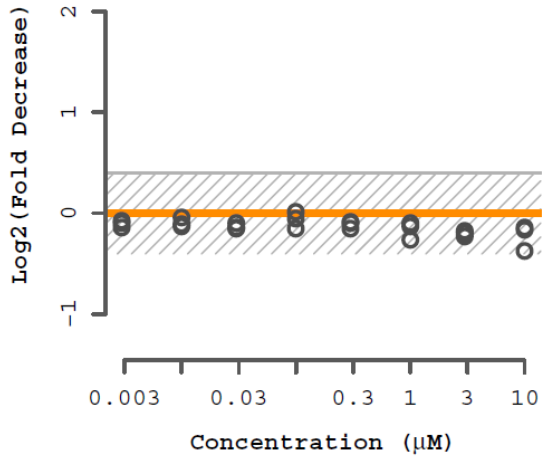
TI = 6.12 µM, CV = no effect

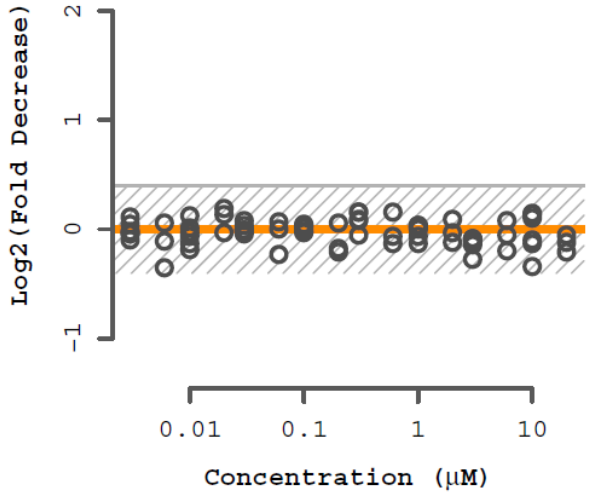
dLEL rat = 20 mg/kg/day (< mLEL)

dLEL rabbit (no ToxRefDB entry)

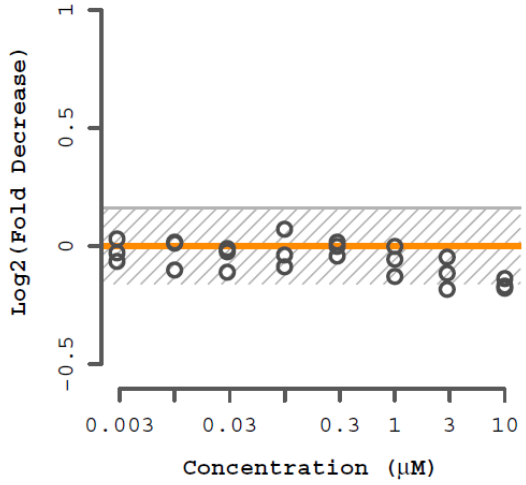
Example: *false negatives (not detected in ToxCast_STM)*

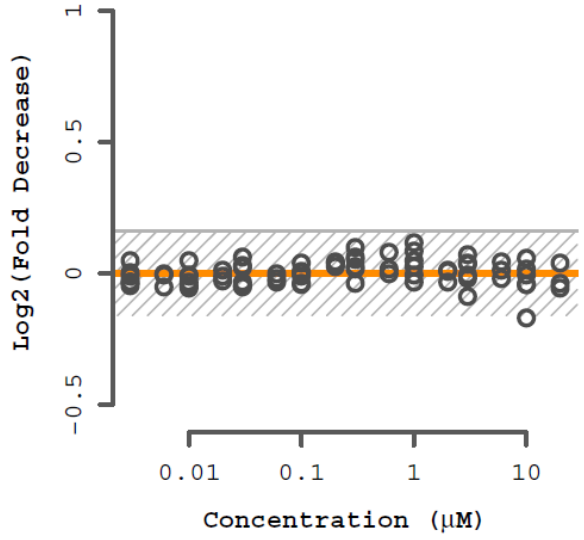
Targeted biomarker (TI)

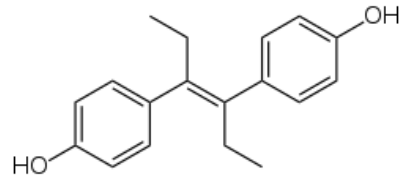




Viable cell number (CV)





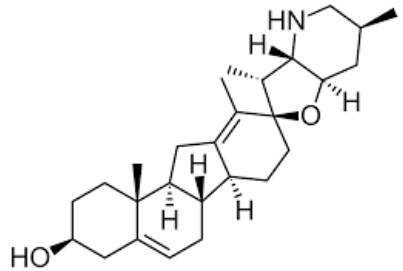


Diethylstilbestrol (DES)

TI = NA, CV = NA

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

(no rabbit data in ToxRefDB)

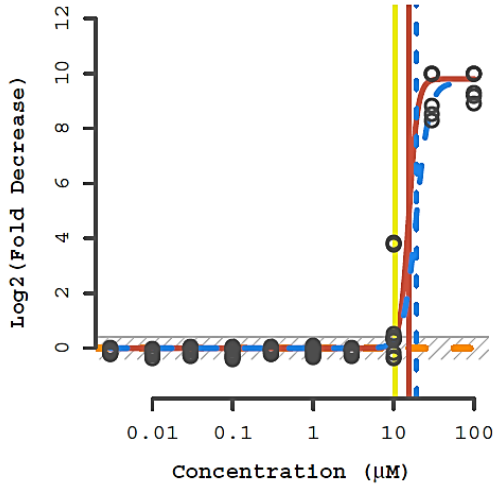


Cyclopamine

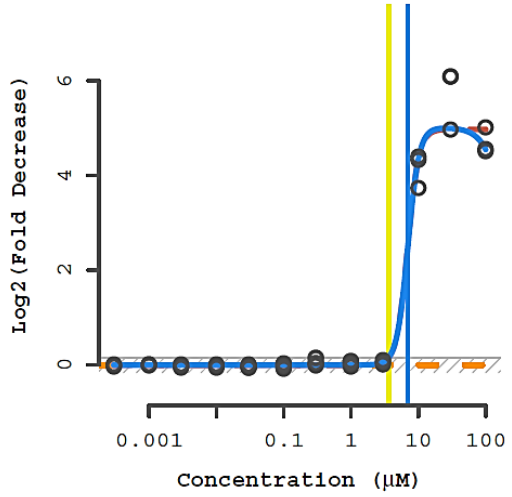
TI = NA, CV = NA

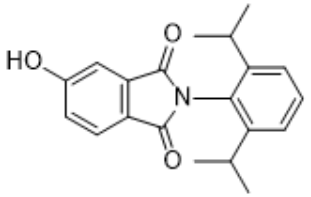
Example: *pharmacological angiogenesis inhibitors*

Targeted biomarker (TI)



Viable cell number (CV)



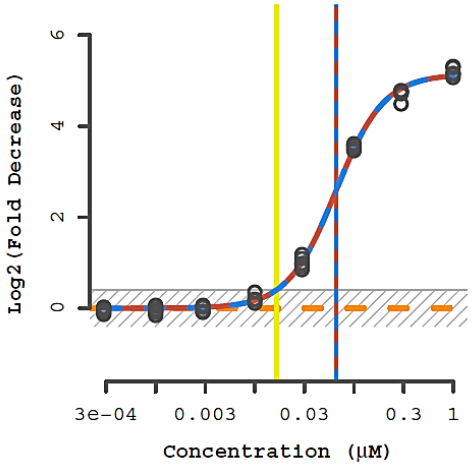


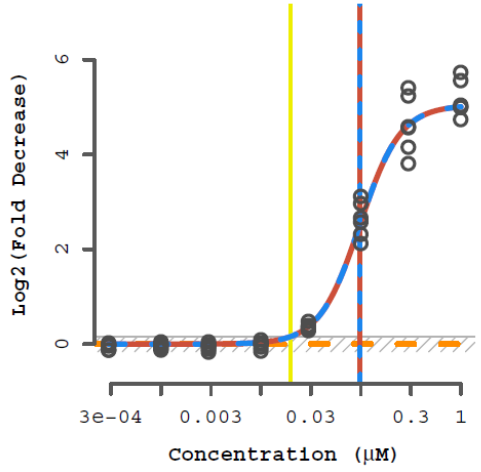
synthetic thalidomide analog

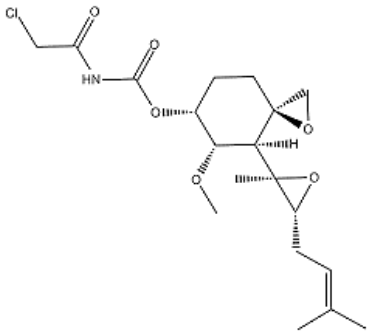
5HPP-33

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)

Does the STM biomarker really predict a teratogenic threshold?

Colleagues at Dow Chemical's Predictive Toxicology group, led by Ed Carney†, tested T.I. predictions for the two structurally diverse angiogenic inhibitors using a 48h rat WEC platform.

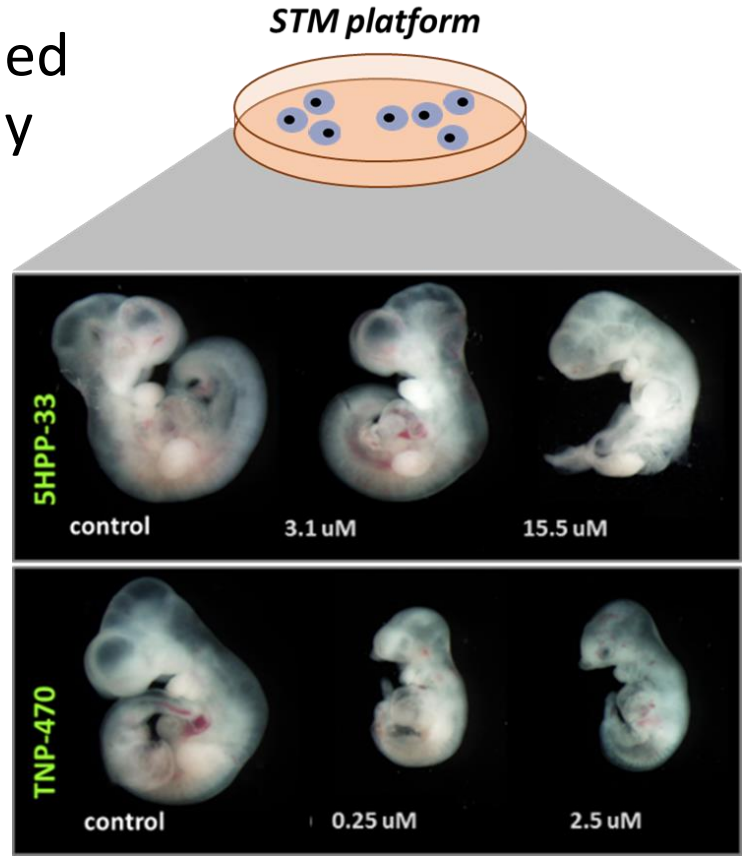
5HPP-33:

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

TNP-470:

- T.I. predicted by hESC was 0.02 µM
- AC50 observed at 0.04 µM (dysmorphogenesis)

Phenotypic consequences differed but the threshold T.I. predicted by the ToxCast_STM data was just under the AC50 observed.

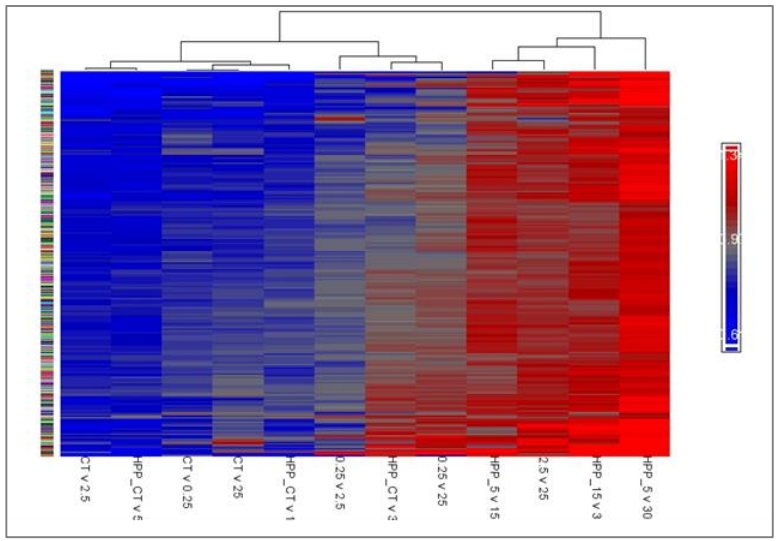


Ellis-Hutchings et al. (2017) Reprod Toxicol

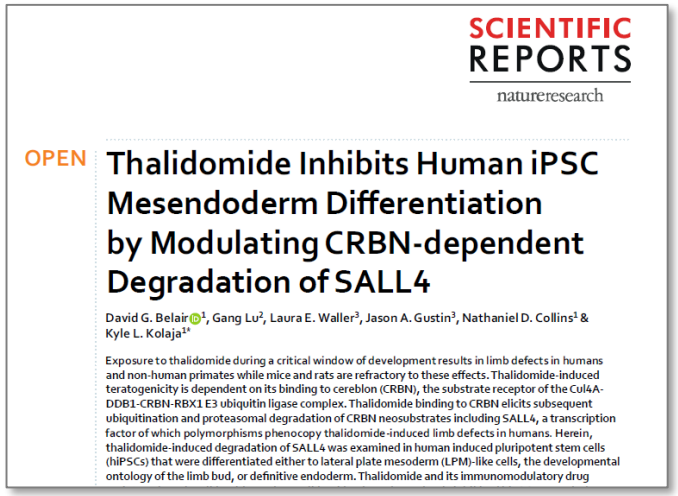
Does the STM biomarker predict something new? *sort of …*

- We used RNA-seq profiling to examine how the exposed embryos reacted to threshold concentrations of 5HPP-33 and TNP-470 after 4 hr exposure in rat WEC.

- Several pathways uniquely affected by each compound, but some in common:
  - *splicesome-RNA metabolism*
  - *proteasome-ubiquitination*



Saili, Franzosa et al. (2019) Curr Opin Tox



SCIENTIFIC REPORTS
nature research

OPEN

Thalidomide Inhibits Human iPSC Mesendoderm Differentiation by Modulating CRBN-dependent Degradation of SALL4

David G. Belair[1], Gang Lu[2], Laura E. Waller[3], Jason A. Gustin[3], Nathaniel D. Collins[1] & Kyle L. Kolaja[1*]

Exposure to thalidomide during a critical window of development results in limb defects in humans and non-human primates while mice and rats are refractory to these effects. Thalidomide-induced teratogenicity is dependent on its binding to cereblon (CRBN), the substrate receptor of the Cul4A-DDB1-CRBN-RBX1 E3 ubiquitin ligase complex. Thalidomide binding to CRBN elicits subsequent ubiquitination and proteasomal degradation of CRBN neosubstrates including SALL4, a transcription factor of which polymorphisms phenocopy thalidomide-induced limb defects in humans. Herein, thalidomide-induced degradation of SALL4 was examined in human induced pluripotent stem cells (hiPSCs) that were differentiated either to lateral plate mesoderm (LPM)-like cells, the developmental ontology of the limb bud, or definitive endoderm. Thalidomide and its immunomodulatory drug

Thalidomide's teratogenicity triggered by cereblon (CRBN) → proteasomal degradation of SALL4 in human iPSC progenitors of limb-bud mesoderm.

Belair et al. (2020) Scientific Reports

Performance check

- 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).
- These performance measures are consistent with the original pharma-trained model [Palmer et al. 2013]

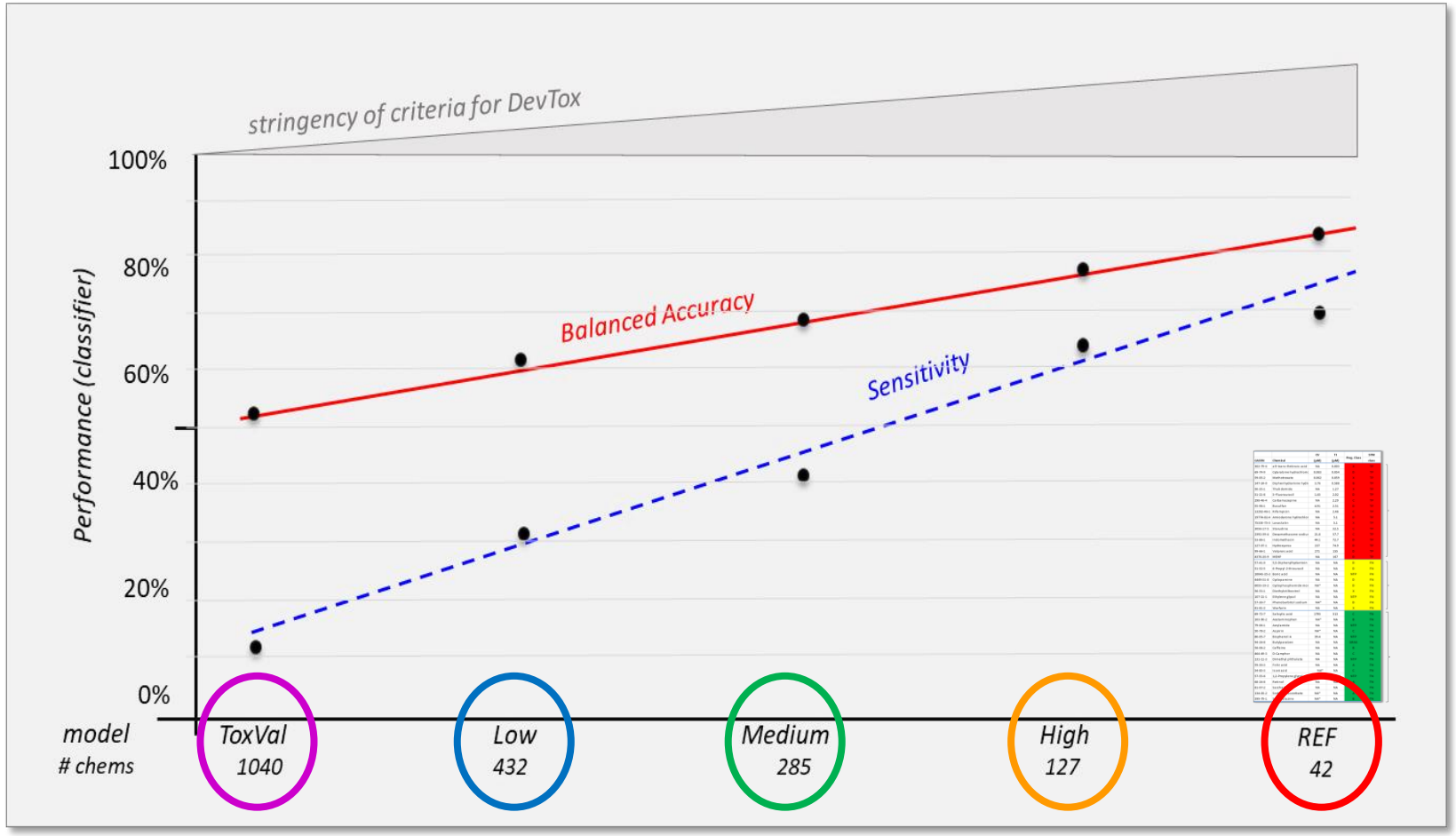
Many assays that have undergone a validation exercise were tested with a limited set of data-rich chemicals, often inflating predictive capacity of 80% or higher. *This situation has hampered regulatory acceptance of individual alternative assays*.

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

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)

Performance evaluation against ToxRefDB chemicals



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 hESC biomarker declines as fetal outcomes gain less concordance between rat-rabbit studies, and concurrent maternal toxicity.

Zurlinden et al. (2020)

Implications of weak sensitivity?

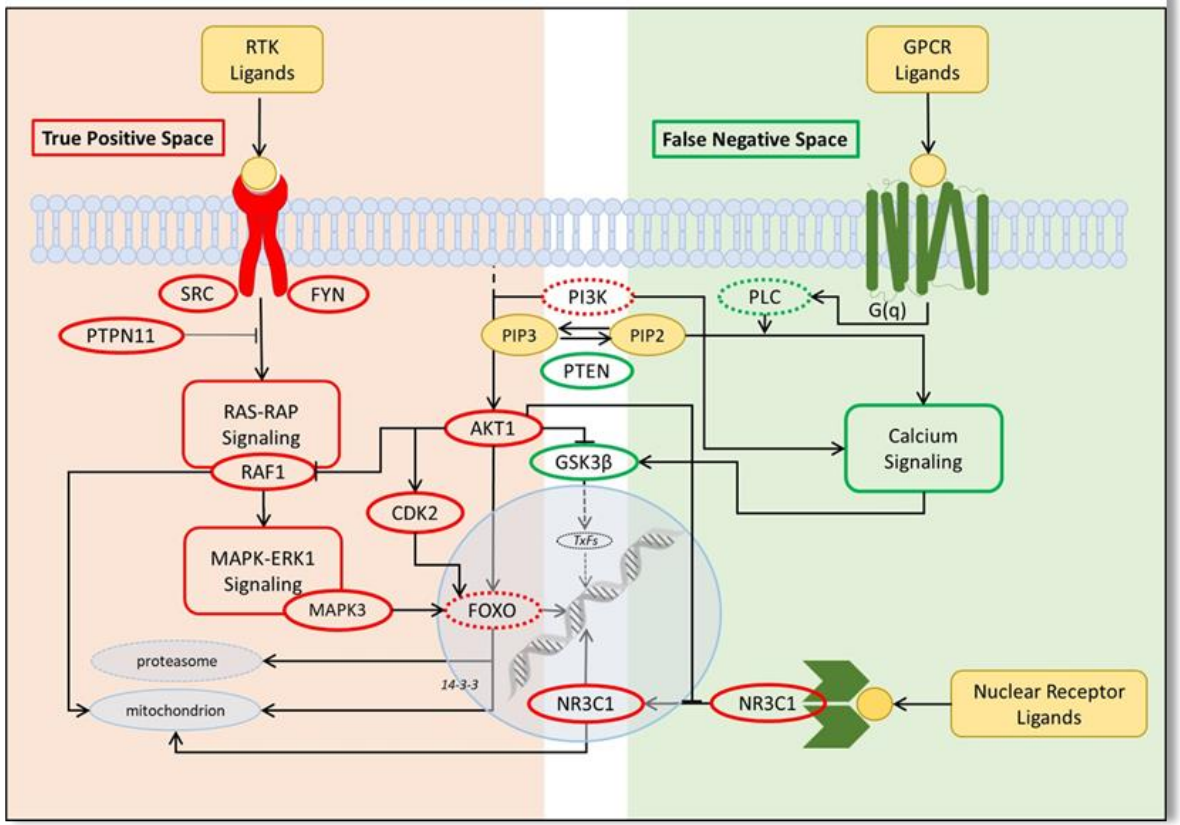
- Predictive power of an assay depends on its biological relevance as well as the standard against which the prediction is validated:
    - ORN/CYSS biomarker may be missing important pathways;
    - pluripotent H9 cells may be missing relevant developmental processes.

With ~1K *in vitro* assays in ToxCast/Tox21, can we use machine learning and mechanistic models to better characterize the biological domain of applicability?

What human relevant pathways are detected or not?

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

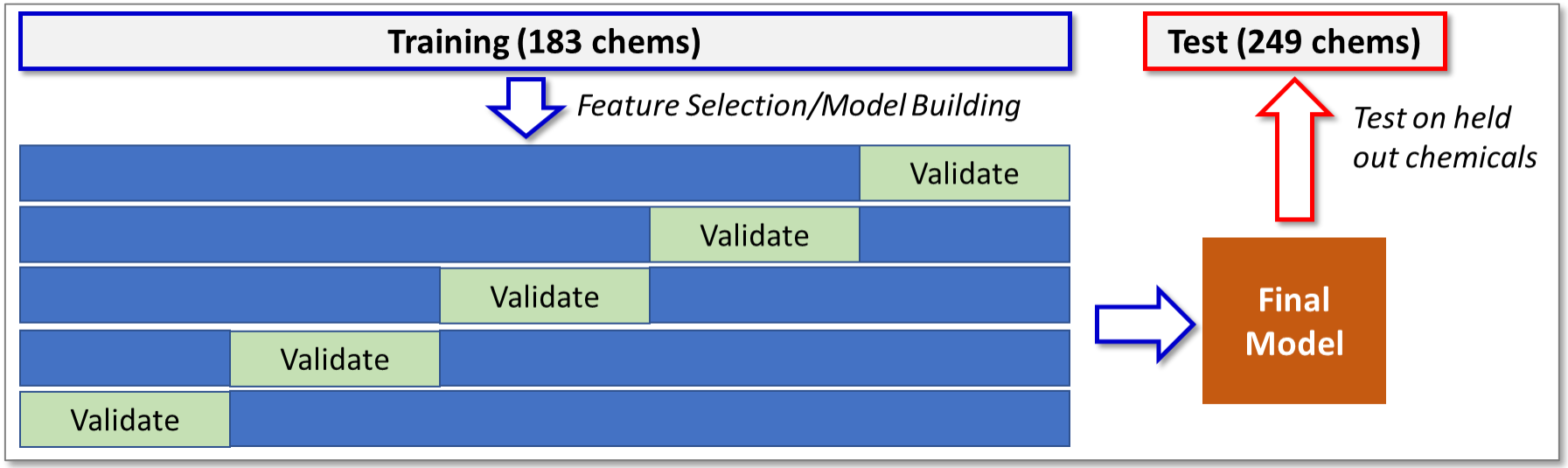


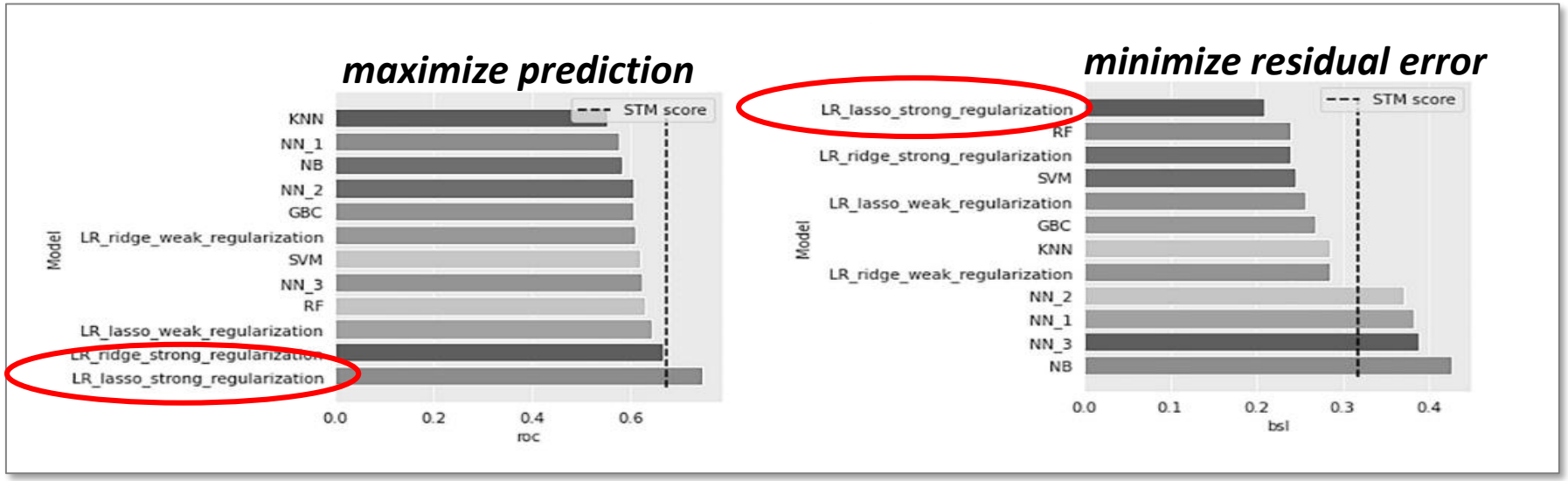
- Mapped ToxCast_STM correlations for well-curated chemicals onto 337 ToxCast_NVS targets;
- flow of regulatory information to AKT/FOXO signaling in the sensitive domain (true positives);
- G(q) signaling and steroid hormone signaling in the insensitive domain (false negatives).

Positive predictivity in the hESC model is driven to FOXO, a key regulator of proteasome-ubiquitination.

Zurlinden et al. (2020) Toxicol Sci

Let's try again … but with all additional ToxCast assays





Unsupervised feature reduction (107)

Algorithms

KNN	K Nearest Neighbors
NB	Naive Bayes
SVM	Support Vector Machine
NN	Neural Network (n hidden layers)
RF	Random Forest
LR	Logistic Regression
GBC	Gradient Boosting Classification

Bayesian regularization - used to shrink assay selection based on residual error.

(----) ROC AUC for DevTox prediction using STM hit call alone.

Zurlinden et al. (manuscript in preparation)

Training model (STM + 7 model)

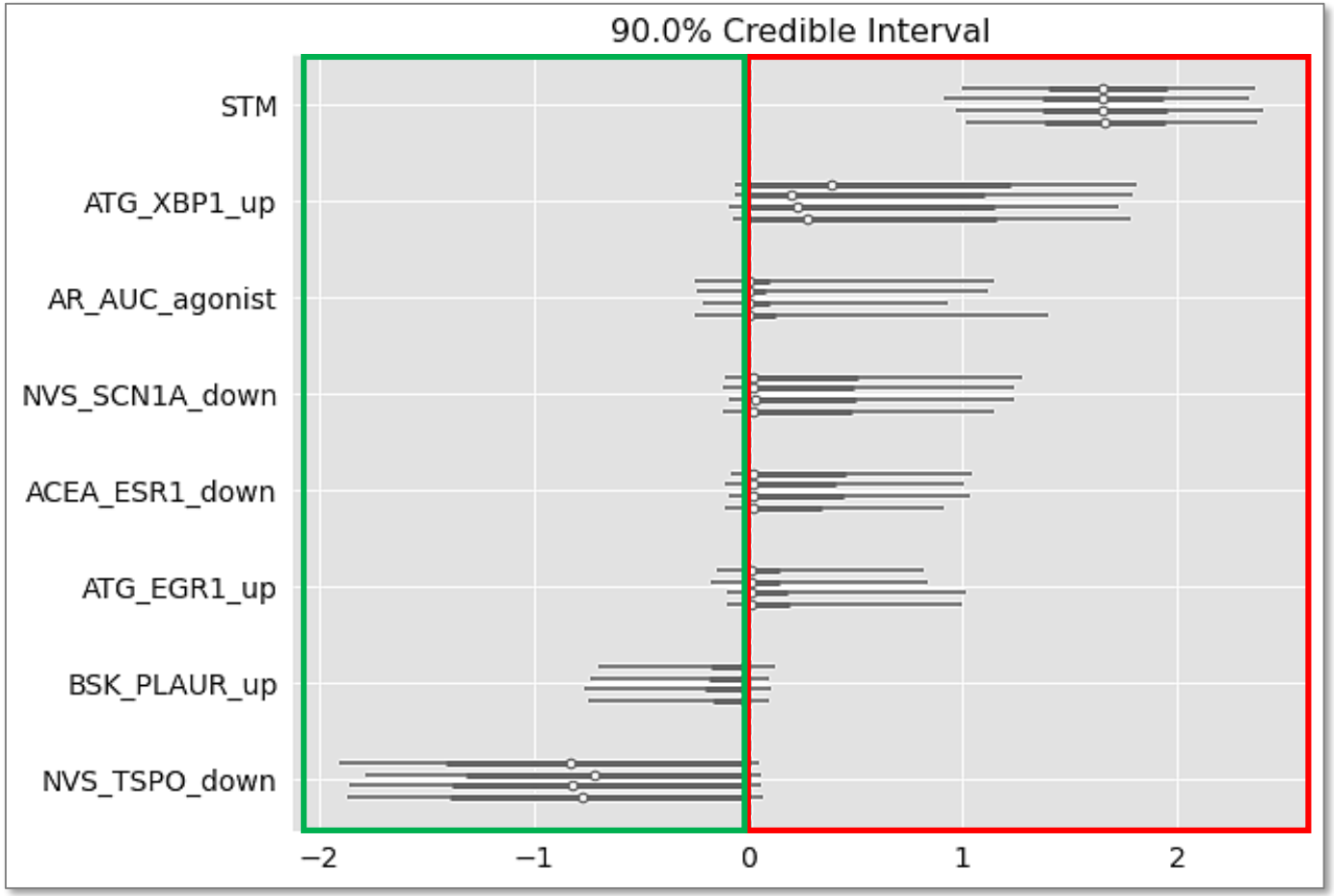
ToxCast_STM is strongest predictor of prenatal developmental toxicity in ToxCast/Tox21;

XBP1 induces UPR and independently targets FOXO1 for proteasomal degradation;

SCN1A picked up some false negatives (e.g., cyclopamine);

Estrogenic and androgenic pathways are outside the STM biological domain;

TSPO mitochondrial 18K translocator that pharmacologically represses XBP1 activation.



Zurlinden et al. (work in progress)

Does the integrated statistical model perform better than ESCs?

Model	Curation	n	sens	PPV	BAC
STM	none	432	0.325	0.803	37.3
	low	432	0.364	0.567	60.4
	medium	285	0.512	0.537	66.5
	high	127	0.667	0.556	72.3
	very high	42	0.654	1.000	82.0
STM+7	train	183	0.593	0.836	77.5
	test	249	0.300	0.469	54.5
Rat subset	90%CI > 0.5	135	0.480	0.783	55.2
	90%CI > 0.7	68	0.736	0.848	60.1
	90%CI > 0.8	23	0.813	0.867	74.6
Rabbit subset	90%CI > 0.5	110	0.429	0.574	50.2
	90%CI > 0.7	58	0.710	0.595	58.3
	90%CI > 0.8	31	0.722	0.650	59.8

Scaling Criteria (ToxRefDB)

- dLEL < 1000 mg/kg/day
- dLEL ≤ 200 mg/kg/day
- dLEL < mLEL, rat OR rabbit
- concordant response, rat AND rabbit
- BM-42 reference

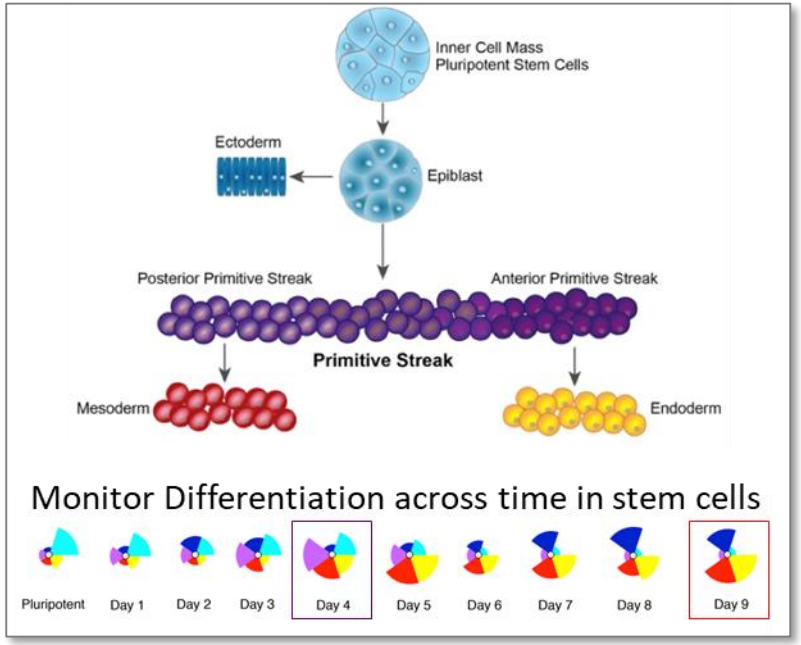
- Train with most confident compound set
- Test on the remainder

A subset of test chemicals with higher confidence in the model can achieve training-level performance.

Zurlinden et al. (work in progress)

Augmentation correlated with the mouse J1 cell (mESC) assay

Goosecoid (GSC) gastrulation marker (day4) and Cardiomyocyte differentiation (day 9)



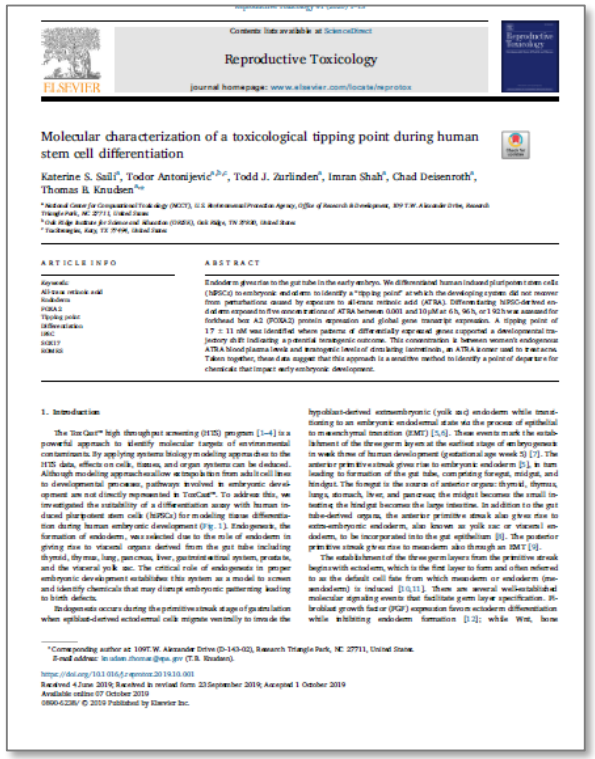
Potency also correlated with effects on mESC growth and differentiation.

Correlation of ToxCast Gene Targets and effects on mESCs

ADRA2C		D4_Cell-*
AHR	GSC_Differentiation-*	D4_Cell-*
AR	GSC_Differentiation-*	D4_Cell-*
CEBPB		D4_Cell-*
CREB3	GSC_Differentiation-*	D4_Cell-*
CYP1A1	GSC_Differentiation-*	
CYP1A2	GSC_Differentiation-*	
CYP2C19	GSC_Differentiation-*	D4_Cell-*
CYP2C9	GSC_Differentiation-*	
CYP2D6	GSC_Differentiation-*	
DT40	GSC_Differentiation-*	D4_Cell-*
EGR1		D4_Cell-*
ELANE	GSC_Differentiation-*	D4_Cell-*
ESR1	GSC_Differentiation-*	D4_Cell-*
FOS	GSC_Differentiation-*	D4_Cell-*
JUN	GSC_Differentiation-*	D4_Cell-*
MAOA		D4_Cell-*
MMP3		D4_Cell-*
MTF1		D4_Cell-*
NFE2L2	GSC_Differentiation-*	D4_Cell-*
NOS1	GSC_Differentiation-*	
NR1I2	GSC_Differentiation-*	D4_Cell-*
NR4A2		D4_Cell-*
OXTR		D4_Cell-*
POU2F1	GSC_Differentiation-*	D4_Cell-*
PPARA		D4_Cell-*
RARA	GSC_Differentiation-*	D4_Cell-*
RORA		D4_Cell-*
RXRB	GSC_Differentiation-*	D4_Cell-*
SCN1A	GSC_Differentiation-*	D4_Cell-*
SMAD1	GSC_Differentiation-*	D4_Cell-*
SOX1		D4_Cell-*
SP1	GSC_Differentiation-*	
SREBF1	GSC_Differentiation-*	D4_Cell-*
TACR1		D4_Cell-*
THRB	GSC_Differentiation-*	
TSPO	GSC_Differentiation-*	
XBP1	GSC_Differentiation-*	D4_Cell-*

Hunter et al. (manuscript in clearance for submission)

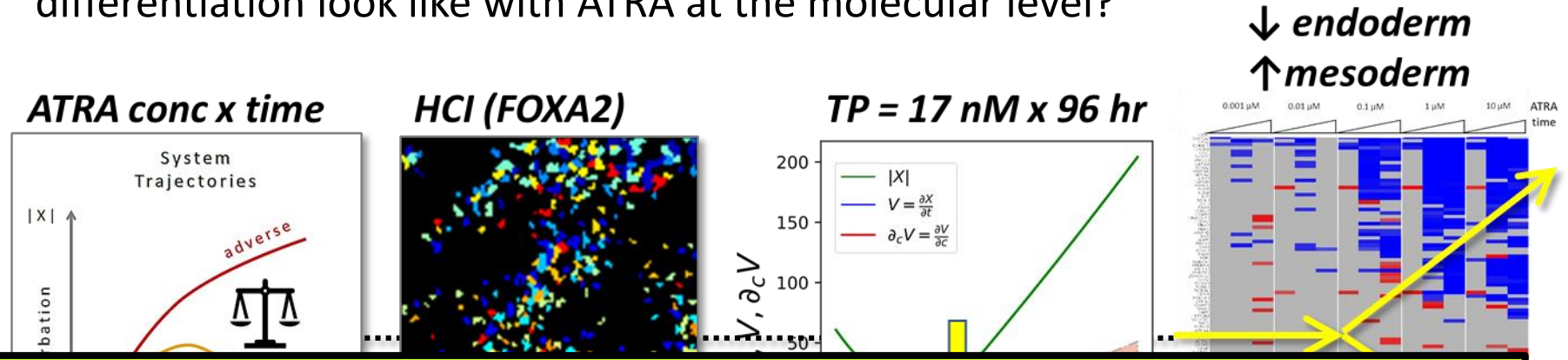
Molecular characterization of a Toxicological Tipping Point



Reproductive Toxicology

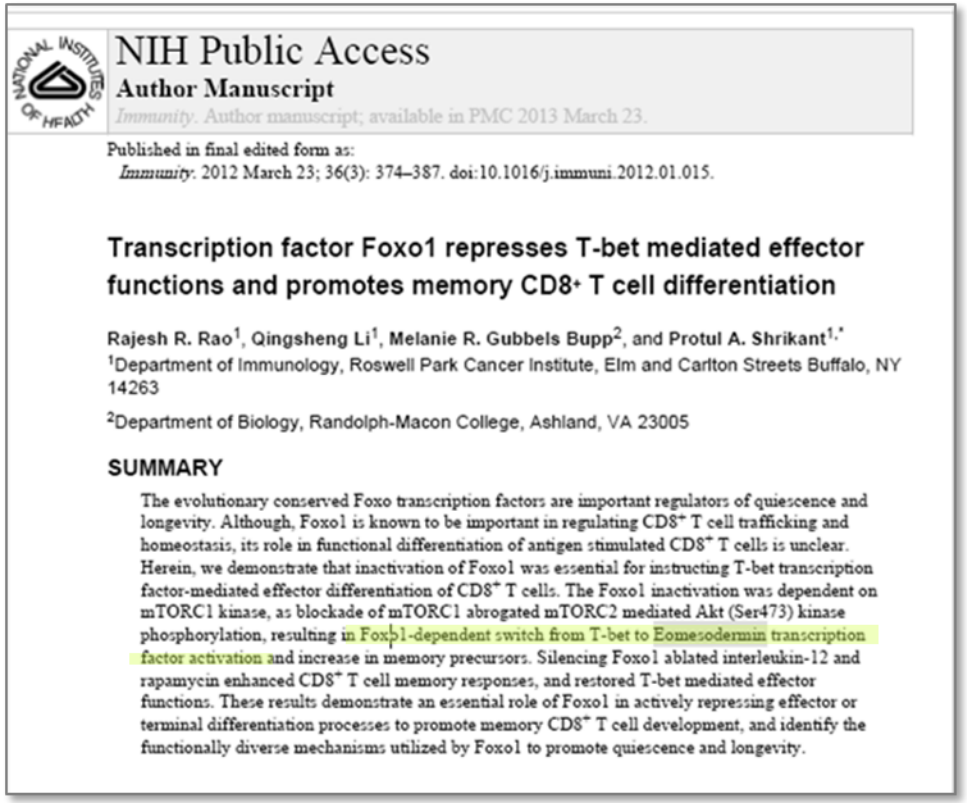
Molecular characterization of a toxicological tipping point during human stem cell differentiation

- hESCs tracked in mesendodermal lineage classified teratogenicity based on SOX17 (87-94% accuracy) [Kameoka et al. 2014];
- *all-trans* retinoic acid (ATRA) is an endogenous signal in mesendodermal patterning (T.I. = 19 nM in hiPSCs [Palmer et al. 2017]);
- what would a 'toxicological tipping point' on hiPSC differentiation look like with ATRA at the molecular level?



The tipping point reflects an imbalance on genes regulated by *Eomesodermin* (*EOMES*), a T-box family member that drives endodermal specification and mesodermal delamination during primitive streak formation (gastrulation).

EOMES is regulated by FOXO: *at least in memory T-cells!*



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Transcription factor Foxo1 represses T-bet mediated effector functions and promotes memory CD8⁺ T cell differentiation

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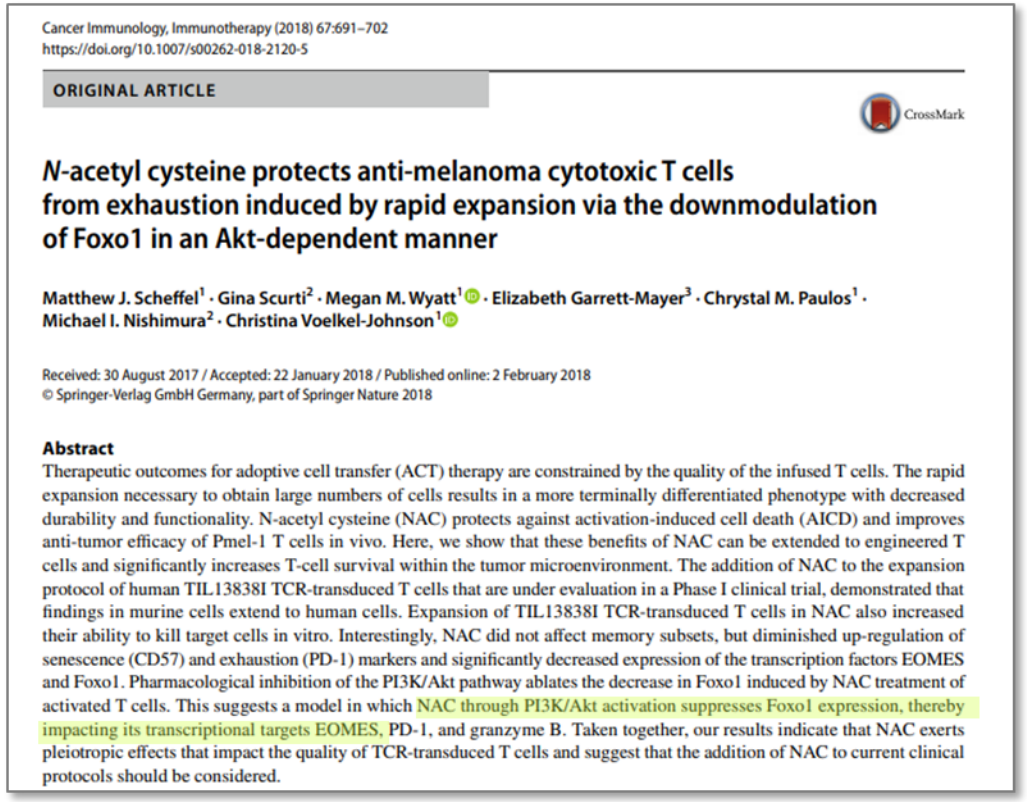
SUMMARY

The evolutionary conserved Foxo transcription factors are important regulators of quiescence and longevity. Although, Foxo1 is known to be important in regulating CD8⁺ T cell trafficking and homeostasis, its role in functional differentiation of antigen stimulated CD8⁺ T cells is unclear. Herein, we demonstrate that inactivation of Foxo1 was essential for instructing T-bet transcription factor-mediated effector differentiation of CD8⁺ T cells. The Foxo1 inactivation was dependent on mTORC1 kinase, as blockade of mTORC1 abrogated mTORC2 mediated Akt (Ser473) kinase phosphorylation, resulting in Foxo1-dependent switch from T-bet to Eomesodermin transcription factor activation and increase in memory precursors. Silencing Foxo1 ablated interleukin-12 and rapamycin enhanced CD8⁺ T cell memory responses, and restored T-bet mediated effector functions. These results demonstrate an essential role of Foxo1 in actively repressing effector or terminal differentiation processes to promote memory CD8⁺ T cell development, and identify the functionally diverse mechanisms utilized by Foxo1 to promote quiescence and longevity.

'… Foxo1-dependent switch from T-bet to Eomesodermin transcription factor activation…



Cancer Immunology, Immunotherapy (2018) 67:691–702
https://doi.org/10.1007/s00262-018-2120-5

ORIGINAL ARTICLE

CrossMark

N-acetyl cysteine protects anti-melanoma cytotoxic T cells from exhaustion induced by rapid expansion via the downmodulation of Foxo1 in an Akt-dependent manner

Matthew J. Scheffel[1] · Gina Scurti[2] · Megan M. Wyatt[1] · Elizabeth Garrett-Mayer[3] · Chrystal M. Paulos[1] · Michael I. Nishimura[2] · Christina Voelkel-Johnson[1]

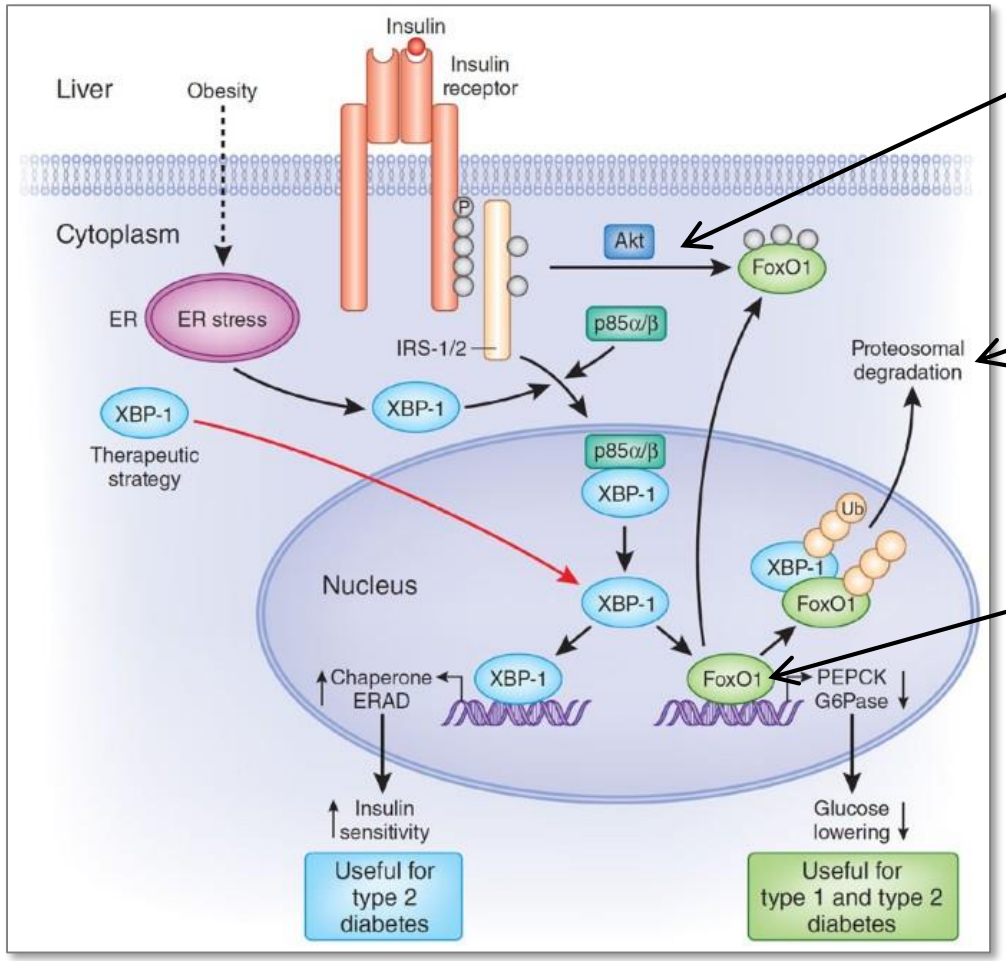
Received: 30 August 2017 / Accepted: 22 January 2018 / Published online: 2 February 2018

Abstract

Therapeutic outcomes for adoptive cell transfer (ACT) therapy are constrained by the quality of the infused T cells. The rapid expansion necessary to obtain large numbers of cells results in a more terminally differentiated phenotype with decreased durability and functionality. N-acetyl cysteine (NAC) protects against activation-induced cell death (AICD) and improves anti-tumor efficacy of Pmel-1 T cells in vivo. Here, we show that these benefits of NAC can be extended to engineered T cells and significantly increases T-cell survival within the tumor microenvironment. The addition of NAC to the expansion protocol of human TIL13838I TCR-transduced T cells that are under evaluation in a Phase I clinical trial, demonstrated that findings in murine cells extend to human cells. Expansion of TIL13838I TCR-transduced T cells in NAC also increased their ability to kill target cells in vitro. Interestingly, NAC did not affect memory subsets, but diminished up-regulation of senescence (CD57) and exhaustion (PD-1) markers and significantly decreased expression of the transcription factors EOMES and Foxo1. Pharmacological inhibition of the PI3K/Akt pathway ablates the decrease in Foxo1 induced by NAC treatment of activated T cells. This suggests a model in which NAC through PI3K/Akt activation suppresses Foxo1 expression, thereby impacting its transcriptional targets EOMES, PD-1, and granzyme B. Taken together, our results indicate that NAC exerts pleiotropic effects that impact the quality of TCR-transduced T cells and suggest that the addition of NAC to current clinical protocols should be considered.

'… PI3K/Akt activation suppresses Foxo1 expression, thereby impacting its transcriptional targets EOMES …

Does regulation of proteasomal function explain hESC positivity?



Ueki and Kadowaki (2011) Nature

AKT/IP3K signaling to FOXO1 was the winning pathway in correlating NVS biochemical targets to STM positivity

RNAseq rat WEC found the proteasome-ubiquitination as an early feature for 2 chemicals predicted positive

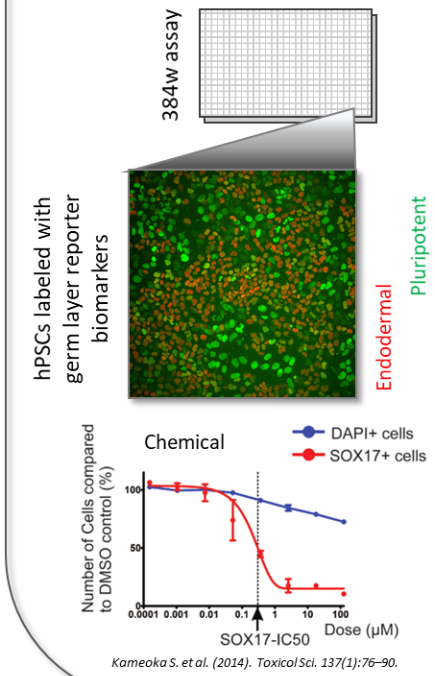
Activation of XBP1 was second only to STM as the winning feature across the broader ToxCast portfolio

HYPOTHESIS: hESC-positivity linked to altered homeostatic control of the FoxO1 axis as a negative regulator of hESC differentiation.

Expanding the molecular landscape of tipping points

Biomarker-based Assay in a hESC Germ Layer Reporter +/- Metabolic Screen

2D HTS screening platform for developing endoderm +/- metabolism screening that utilizes a transgenic hESC line with fluorescent protein reporters for representative germ layer genes.



Kameoka S. et al. (2014). Toxicol Sci. 137(1):76–90.

hPSCs seeded in 2D 384-well plate.

Definitive endoderm media is added to induce differentiation.

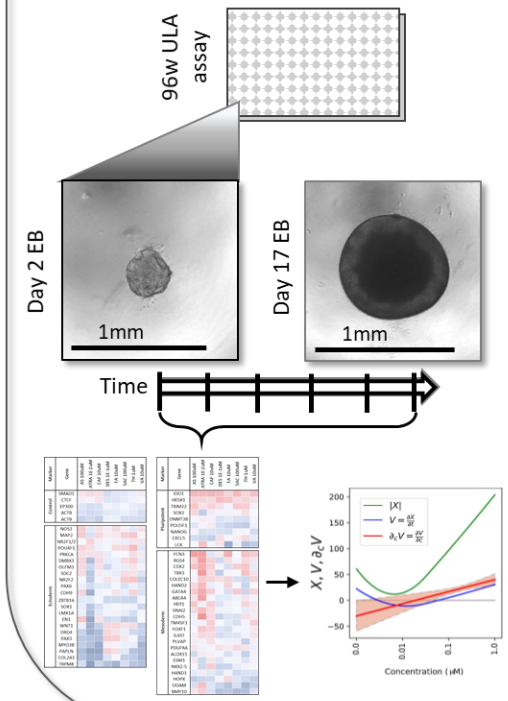
Chemical exposure of hPSCs +/- additional hepatic metabolism method.

High content imaging to identify percentage of differentiated (SOX17+) and pluripotent cells.

Chemicals that increase or decrease hPSCs endoderm differentiation are prioritized for further evaluation.

Gene Expression Signature-based hEBT in hiPSC

Prioritized chemicals undergo gene expression analysis in a 3D organotypic culture model to provide temporal information on lineage deviation and data streams for calculating tipping point thresholds.



Saili K. et al. (2020). Rep. Tox. 91(1):1-13.

hPSCs seeded and aggregated in 3D 96-well ultra low adherence plate.

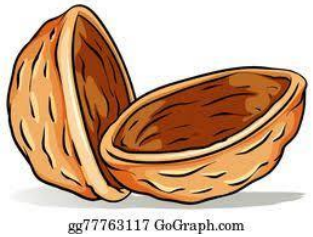
Spontaneous germ layer differentiation of hPSCs in embryoid body (EB).

Time-course chemical exposure acquiring samples over the course of multiple weeks.

Multi-lineage qPCR gene signature revealing perturbations to differentiation pathways.

Temporal and mechanistic evaluation identify a point of departure for chemicals that impact early embryonic development.

John Gamble, Chad Deisenroth (work in progress)



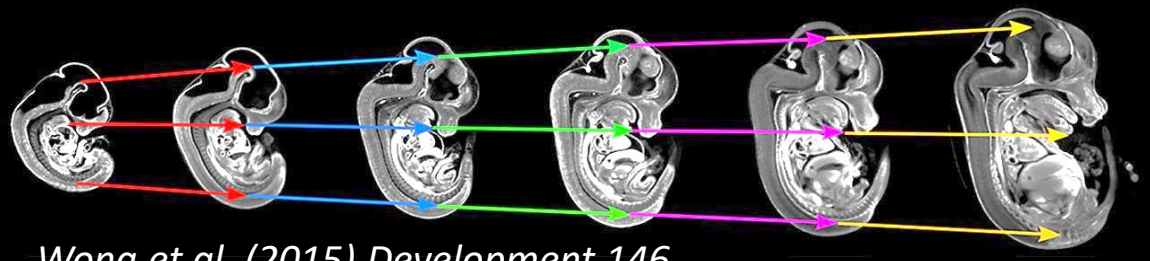
Computational models for NAMs:

predicting developmental toxicity and translation to human pregnancy

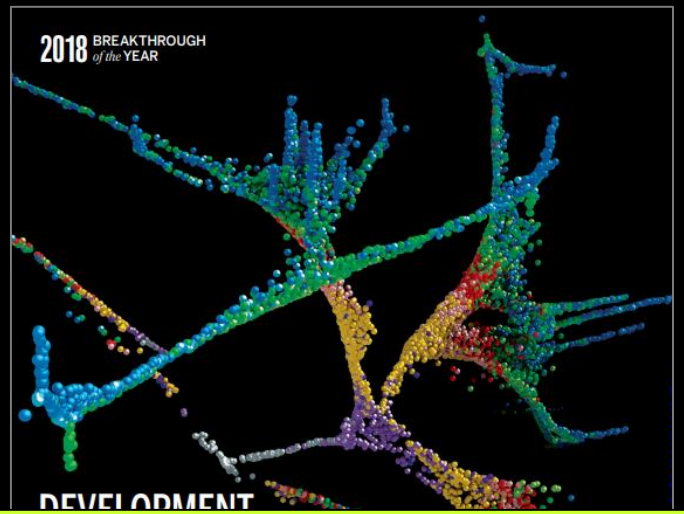
- HTS data-driven models can confidently predict developmental toxicity potential in pregnant rat/rabbit study designs; but …
- … there are limitations of mESC and hESC platforms for classifying teratogenic across a mechanistically diverse landscape of chemicals (pathways, metabolism, complexity, …);
- bringing embryology into the fold will further improve mechanistic understanding in translating NAM data to probabilistic effects and early lifestage phenotypes.

I'll end with two new ways to evaluate the embryo suggesting why sophisticated computer models are needed in the immediate future.

Cell State Manifold: *single-cell transcriptome profiling*



Wong et al. (2015) Development 146

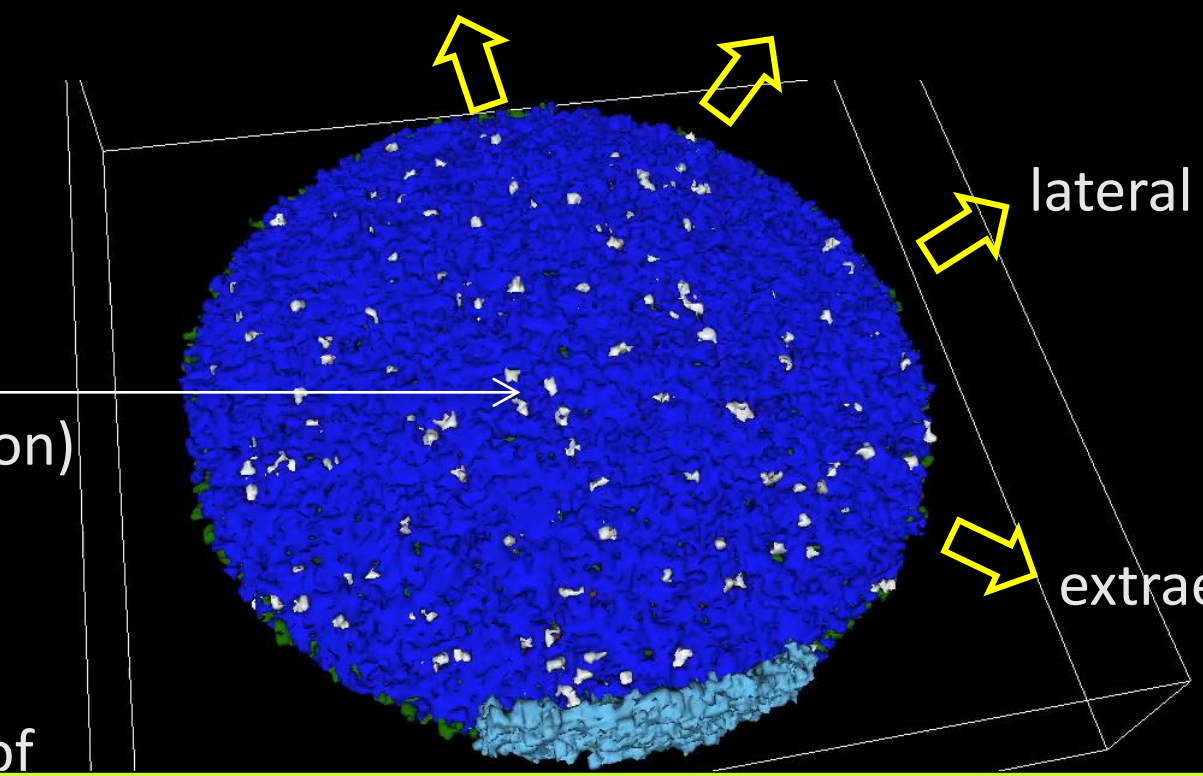


Cao et al. (2019) Nature 566

Video from Malte Spielmann, with thanks

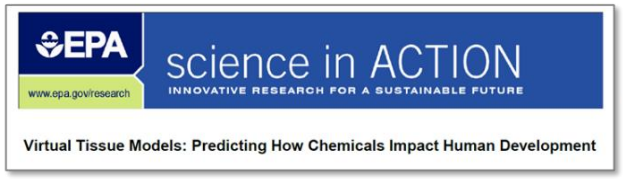
A key challenge for science and technology is to critically define sentinel cells in a perturbed system that propagate chemical injury to toxicological tipping point, ultimately manifesting as a structural defect or altered physiological function.

Epiblast: *spatial dynamics of mesodermal birth*



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

Acknowledgements



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