Computer Simulation of Developmental Processes and Toxicities

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for Modeling Developmental and Reproductive Toxicity [ITS]

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Prenatal Developmental Toxicity

- Developmental and Reproductive Toxicity testing (DART) is important for assessing hazards of drug/chemical exposure to formative processes during early life-stages.

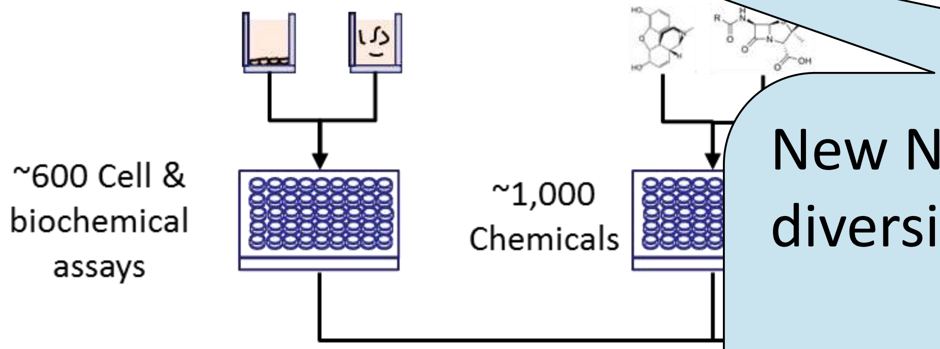
- Prenatal testing (OECD TG 414) entails exposing pregnant rats/rabbits during organogenesis and evaluating adverse outcomes to fetal growth and development.

- Traditional test methods lack throughput and mechanistic support needed for chemicals management under TSCA reform.

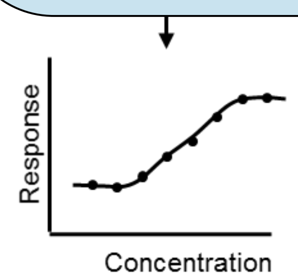
- A compendium of *in vitro* data from ToxCast/Tox21 high-throughput screening (HTS) programs is available for predictive toxicology.

Shifting to Molecular/Pathway Approaches

ToxCast | Tox21



Set	Chemicals	Assays	Completion
ToxCast Phase I	293	~600	2011
ToxCast Phase II	767	~600	2013
ToxCast Phase III	1001	~100	Ongoing
E1K (endocrine)	880	~50	2013



New NCCT contracts in 2014 to increase the diversity of assays used for assessing DevTox:

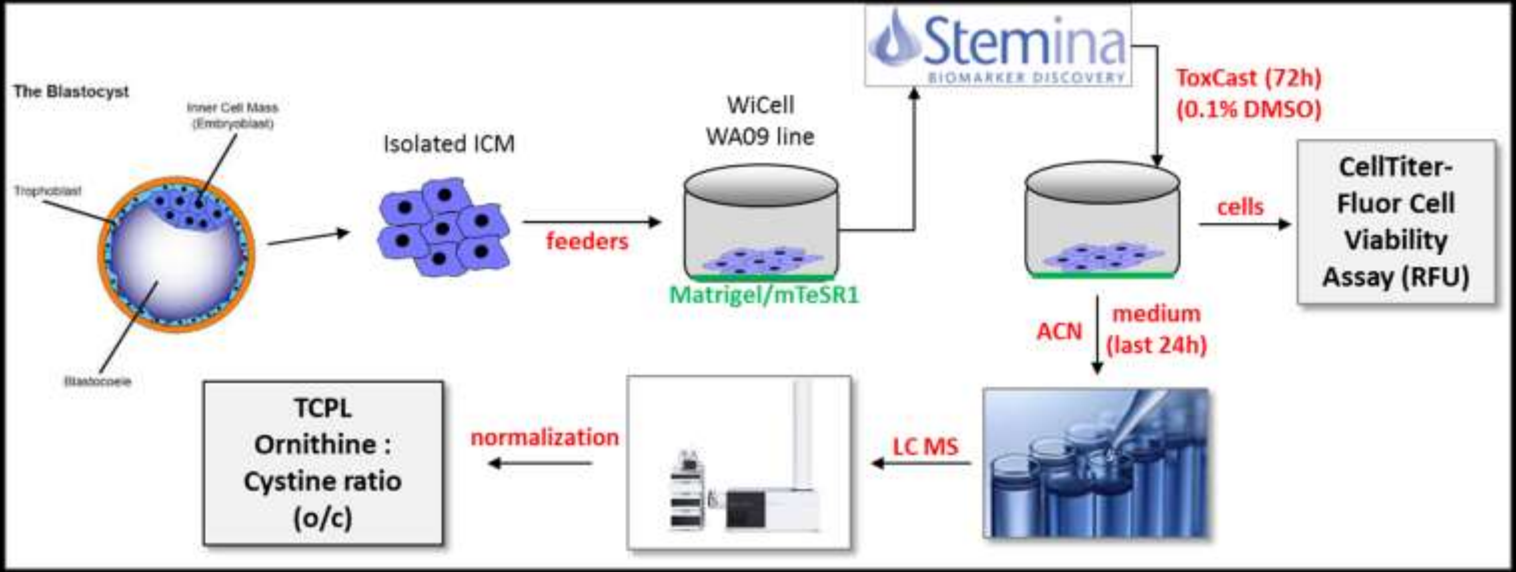
- Stemina Biomarker Discovery
- ArunA Biomedical
- Odyssey Thera, Inc.
- VALA Sciences

SOURCE: Rusty Thomas, Director - NCCT

In a nutshell …

- The devTOX quickPredict platform (Stemina) is a human pluripotent stem cell-based *in vitro* assay used to assess compounds for potential developmental toxicity.
- We screened 1066 ToxCast chemicals to derive an exposure-based potential for developmental toxicity and entered the data into the ToxCast pipeline (tcpl).
- Cellular agent-based models built from the known embryology recapitulate complex signaling networks and simulate critical developmental transitions (and defects).
- Simulation models are numerically responsive to perturbation, hence amenable to for translating HTS bioactivity data into mechanistic prediction models of toxicity.

devTOXqP (quickPREDICT) platform



- WA09 (H9) line is a hESC line approved for federally-funded research and commercially obtained from WiCell Research Institute, Inc (WA09).
- Considered a "gold standard" by stem cell researchers due to stability (normal female karyotype) and long-standing use (hundreds of publications).
- H9 cells maintained in undifferentiated (pluripotent) state in a 96-well format and exposed to chemicals for 3-days; media from last 24h analyzed by LC-MS.

ToxCast Profiling in the STM Platform

- Target exposure range based on ToxCast's cytotox burst [Judson et al. (2016) Tox Sci], compound availability, and/or compound insolubility in DMSO.

- Individual plate references used Methotrexate (MTX) for negative- (5 nM) and positive- (1 µM) responses; and vehicle control (0.1% DMSO) for plate-level normalization.

- Media from last 24h exposure processed for metabolite analysis by HILC-HRMS (high-resolution mass spectroscopy).

- Ornithine (ORN) to Cystine (CYSS) ratio in the conditioned medium ('secretome') is the targeted biomarker [Palmer et al. 2013].

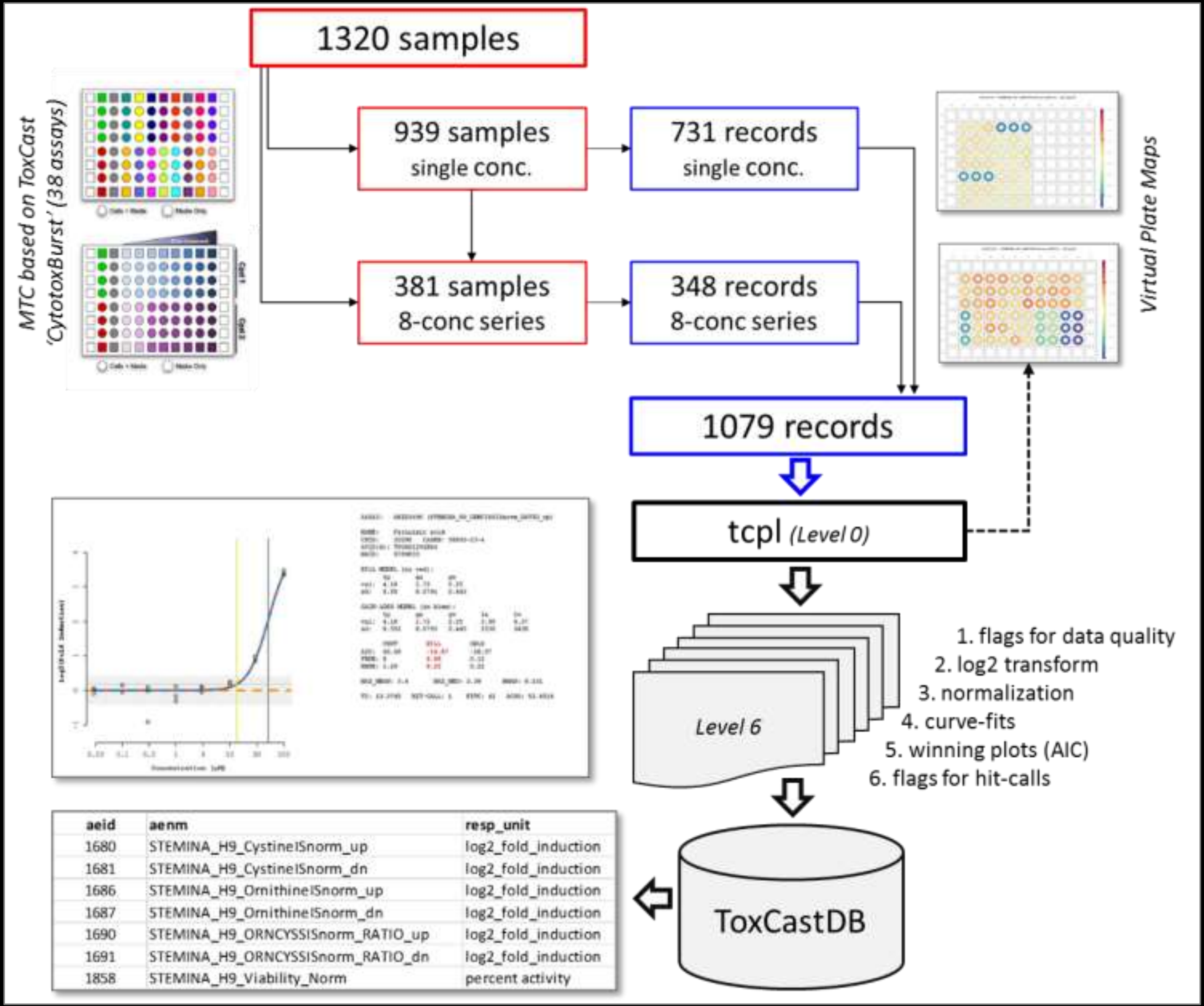
Why does the ORN/CYSS balance matter?

ORN utilized by mitochondria in the 'ornithine cycle' during pyrimidine synthesis; cellular release likely a stress signal.

CYSS from the medium utilized for glutathione synthesis in the redox cycle; reduced uptake likely a stress signal.

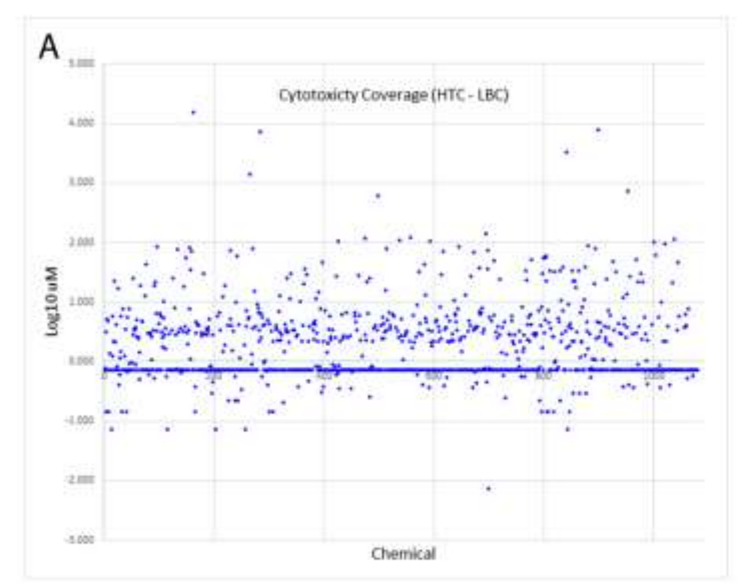
ORN/CYSS falling below 0.88 is predictive of dTP; driven primarily by ORN release.

Strategy

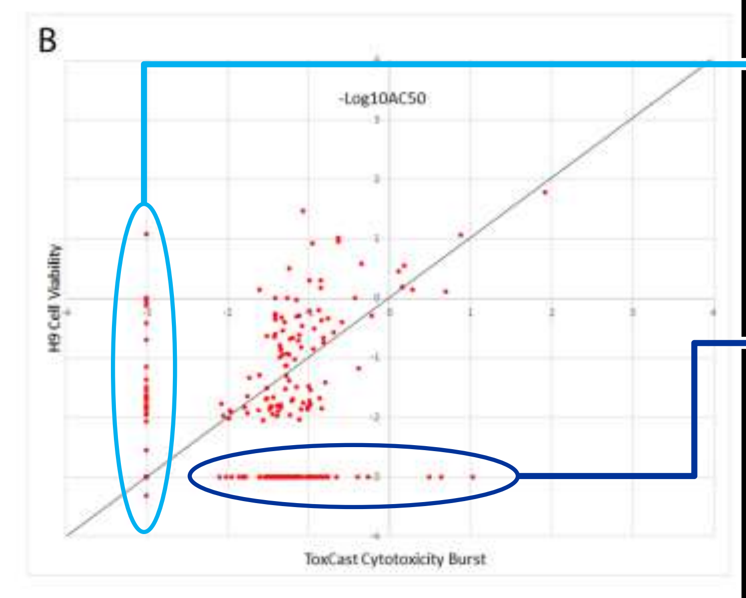


aeid	aenm	resp_unit
1680	STEMINA_H9_CystineISnorm_up	log2_fold_induction
1681	STEMINA_H9_CystineISnorm_dn	log2_fold_induction
1686	STEMINA_H9_OrnithineISnorm_up	log2_fold_induction
1687	STEMINA_H9_OrnithineISnorm_dn	log2_fold_induction
1690	STEMINA_H9_ORNCYSSISnorm_RATIO_up	log2_fold_induction
1691	STEMINA_H9_ORNCYSSISnorm_RATIO_dn	log2_fold_induction
1858	STEMINA_H9_Viability_Norm	percent activity

H9 viability versus ToxCast (38 cytotoxicity/cell stress assays)



Highest Tested Concentration (HTS) at or above the lower bounds of the ToxCast cytotoxicity point (TCB minus three times the global MAD) [Judson et al. (2016)] for most chemicals.

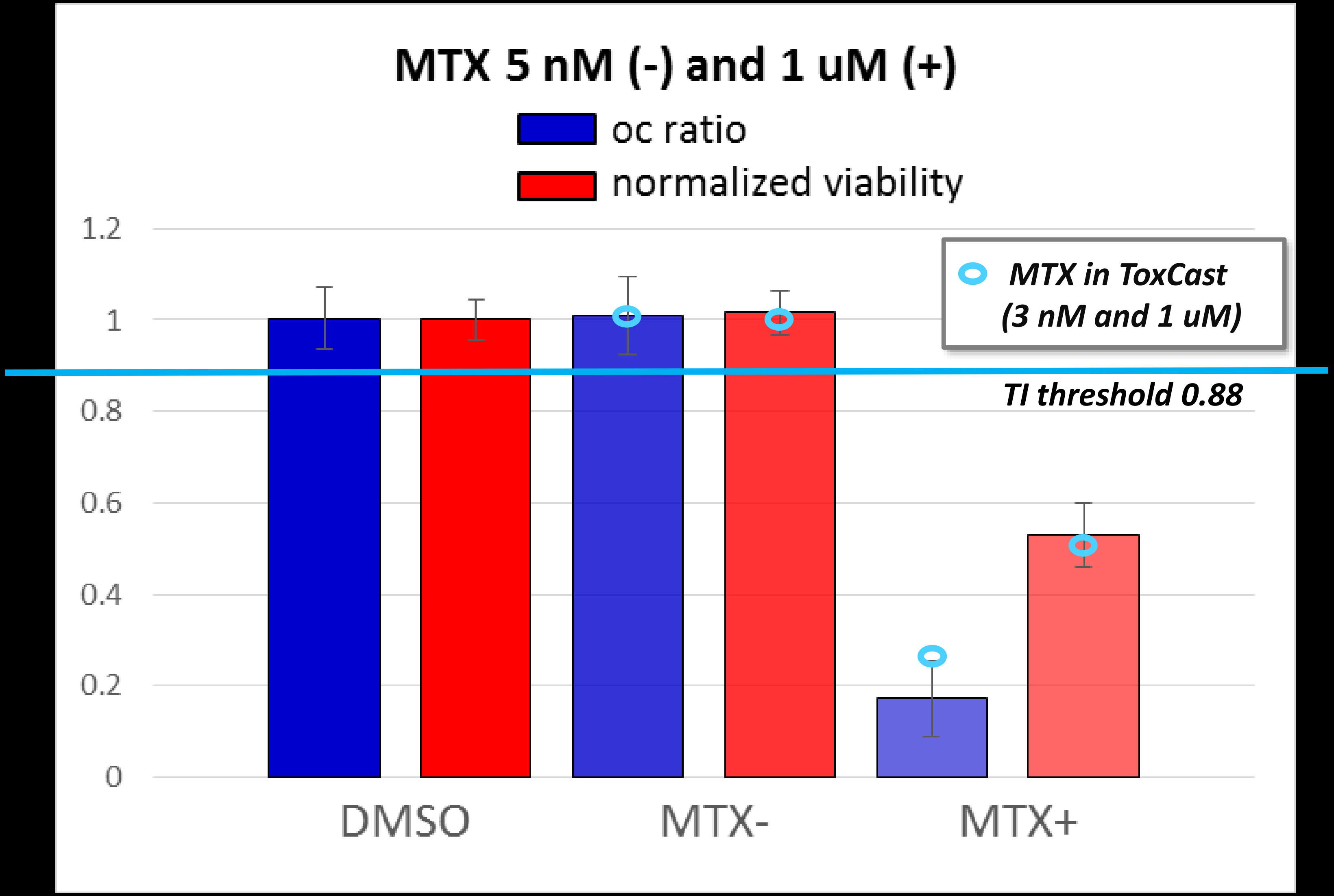


H9 cell viability more sensitive than ToxCast Cytotoxic Burst.

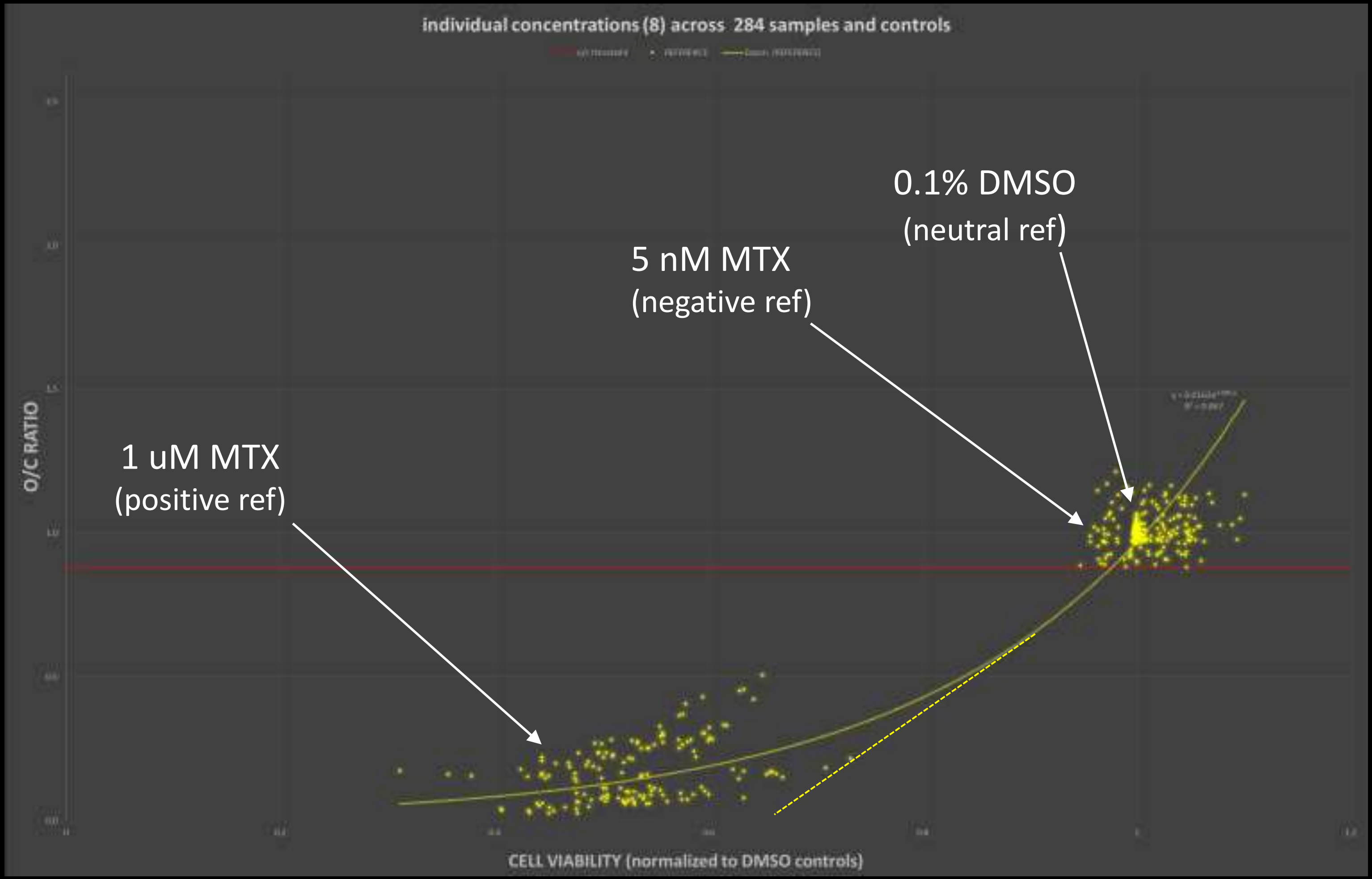
H9 cell viability less sensitive than ToxCast Cytotoxic Burst.

Plate Controls (Level-0 data)

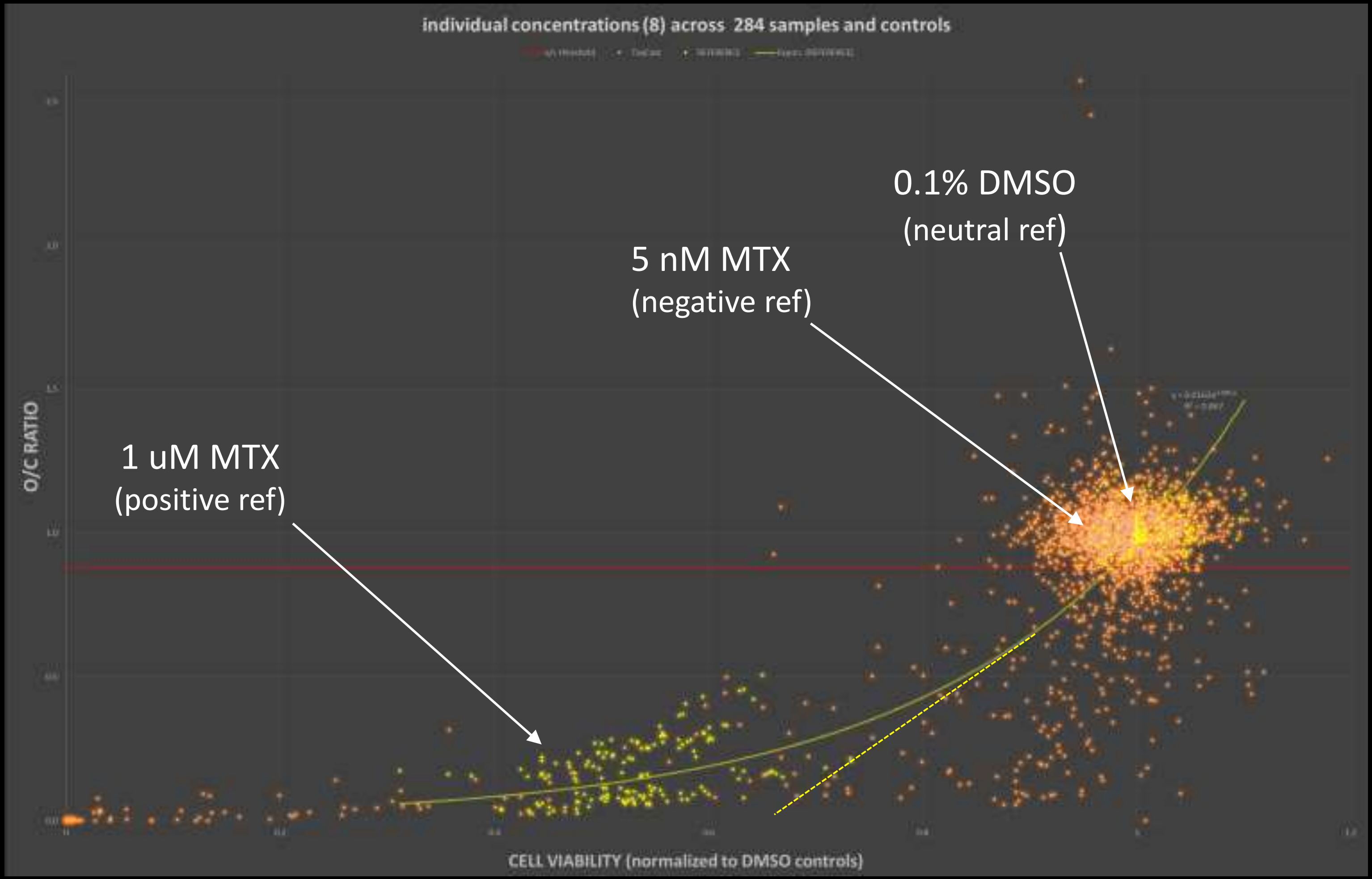
DMSO (0.1%, n = 846, 857), MTX-negative (5 nM, n = 425, 429) and MTX-positive (1 uM, n = 424, 429).



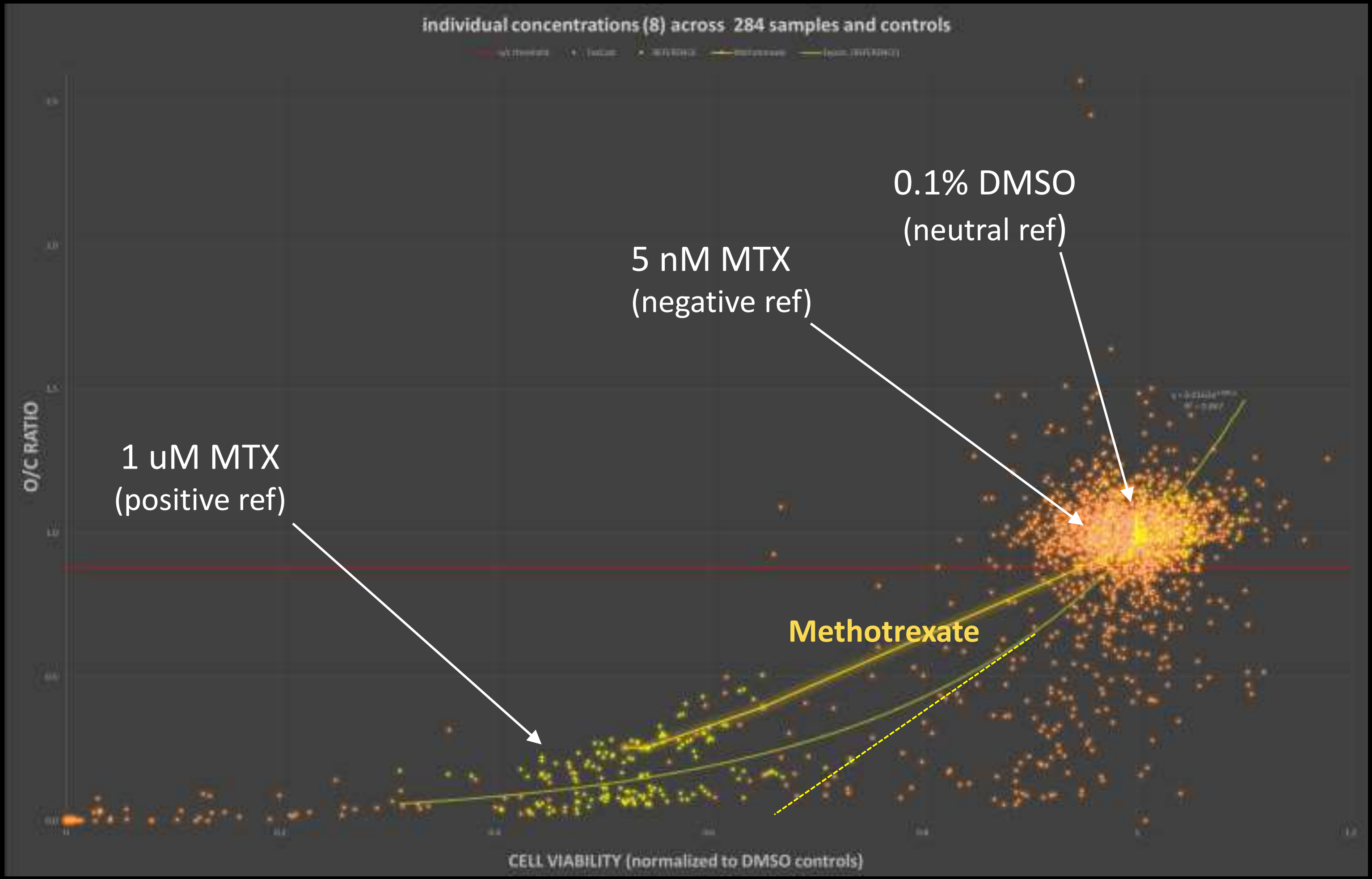
Targeted Biomarker (o/c ratio in the medium) versus cell viability: PLATE CONTROLS



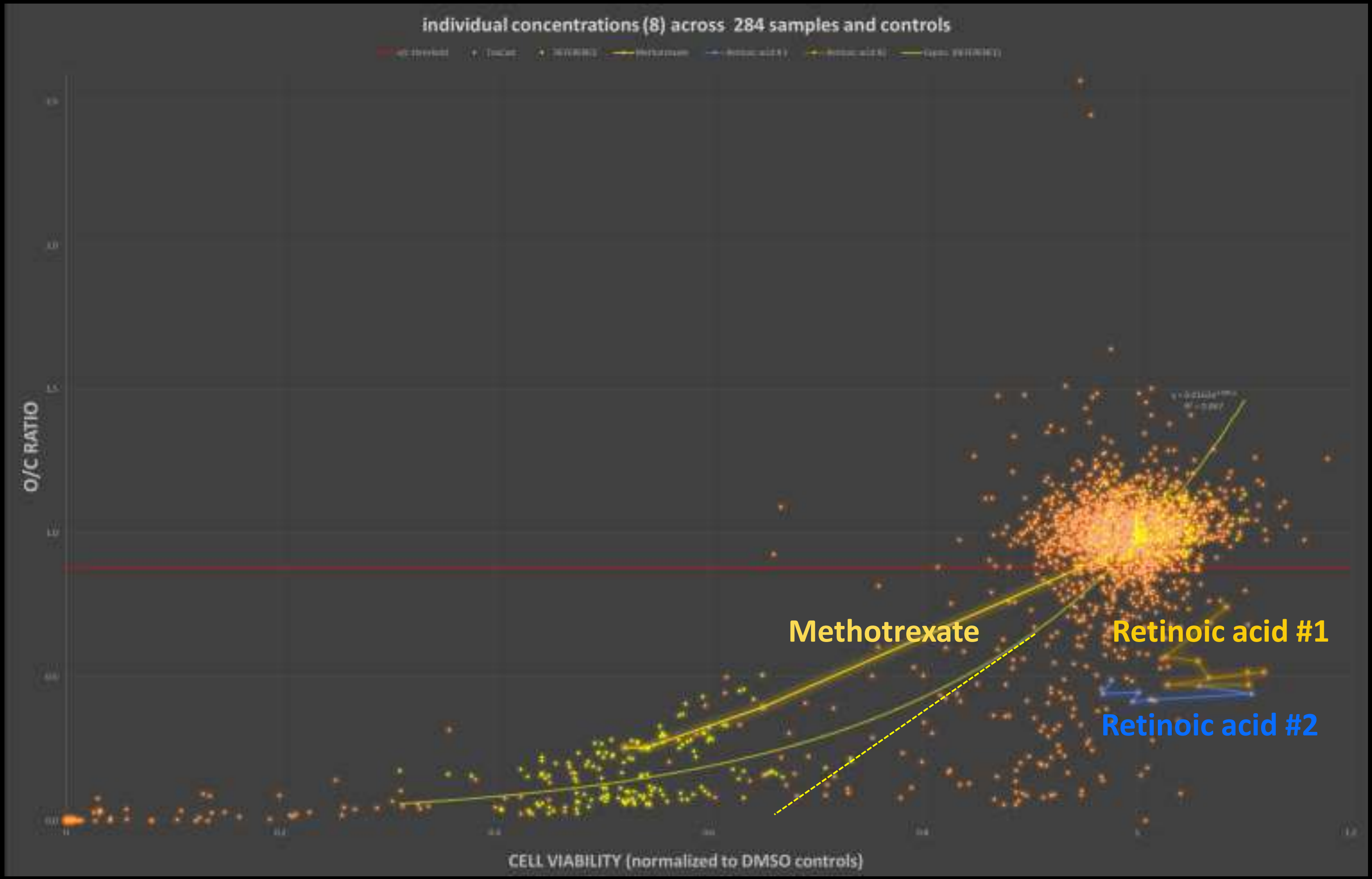
Targeted Biomarker (o/c ratio in the medium) versus cell viability: TOXCAST SAMPLES



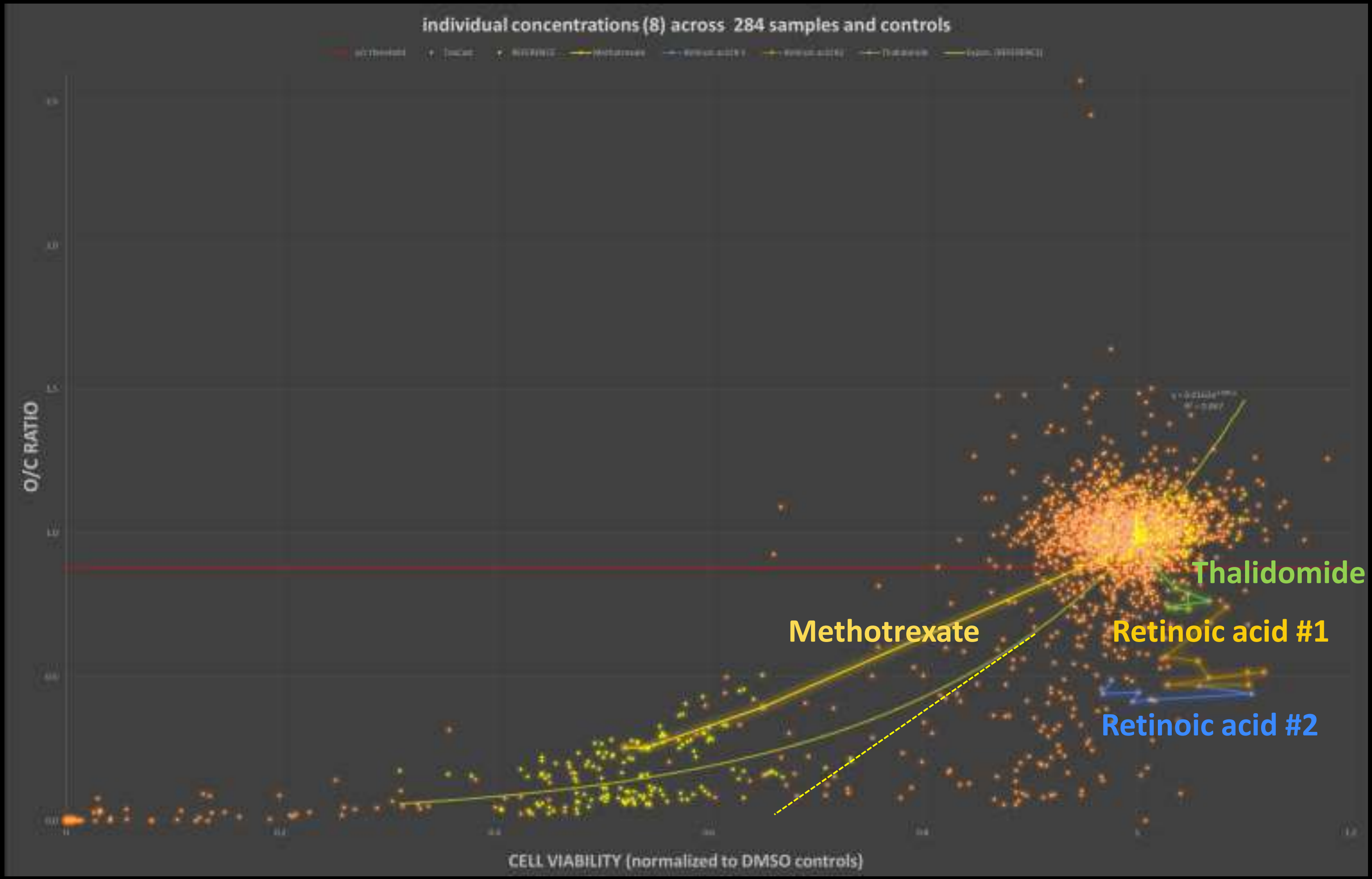
Targeted Biomarker (o/c ratio in the medium) versus cell viability: Methotrexate



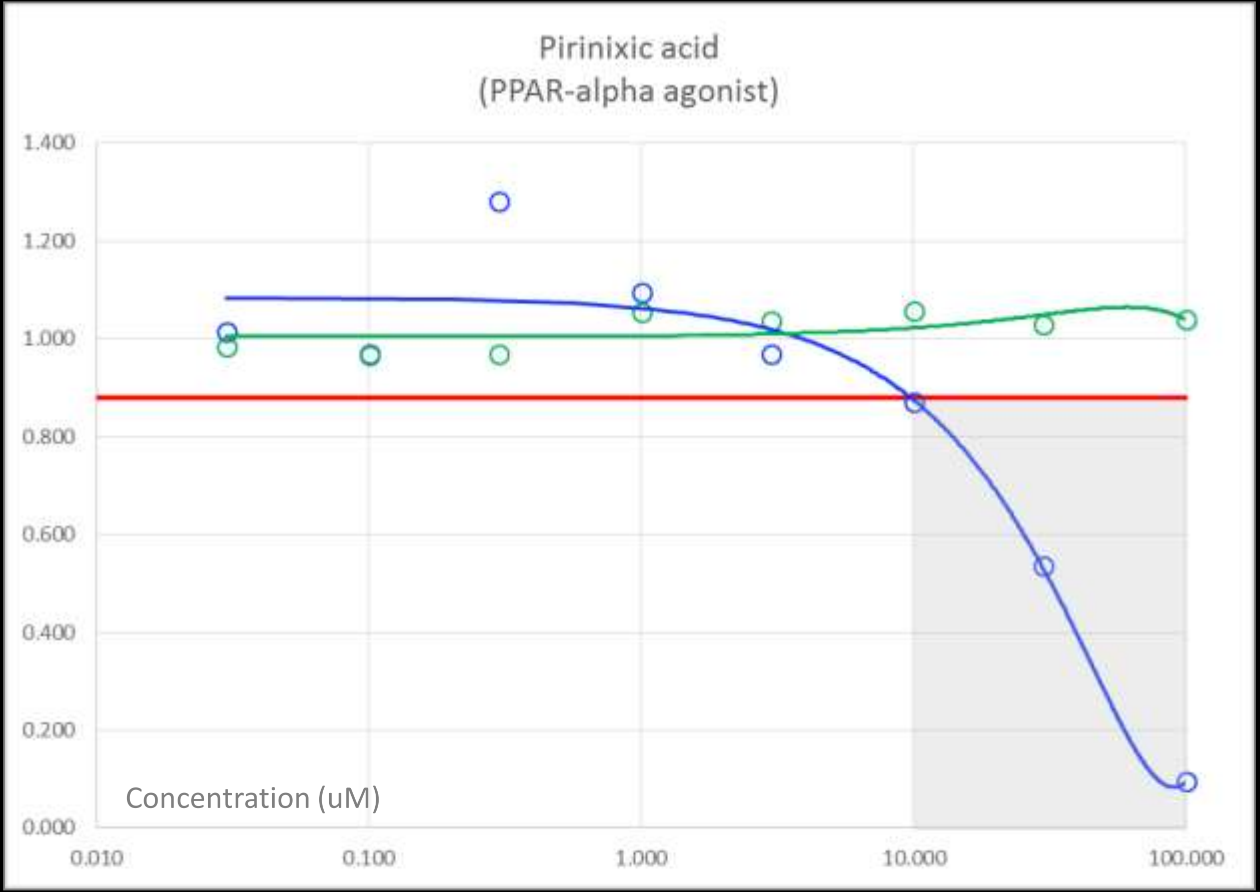
Targeted Biomarker (o/c ratio in the medium) versus cell viability: trans-Retinoic acid



Targeted Biomarker (o/c ratio in the medium) versus cell viability: Thalidomide



How Stemina interprets this assay



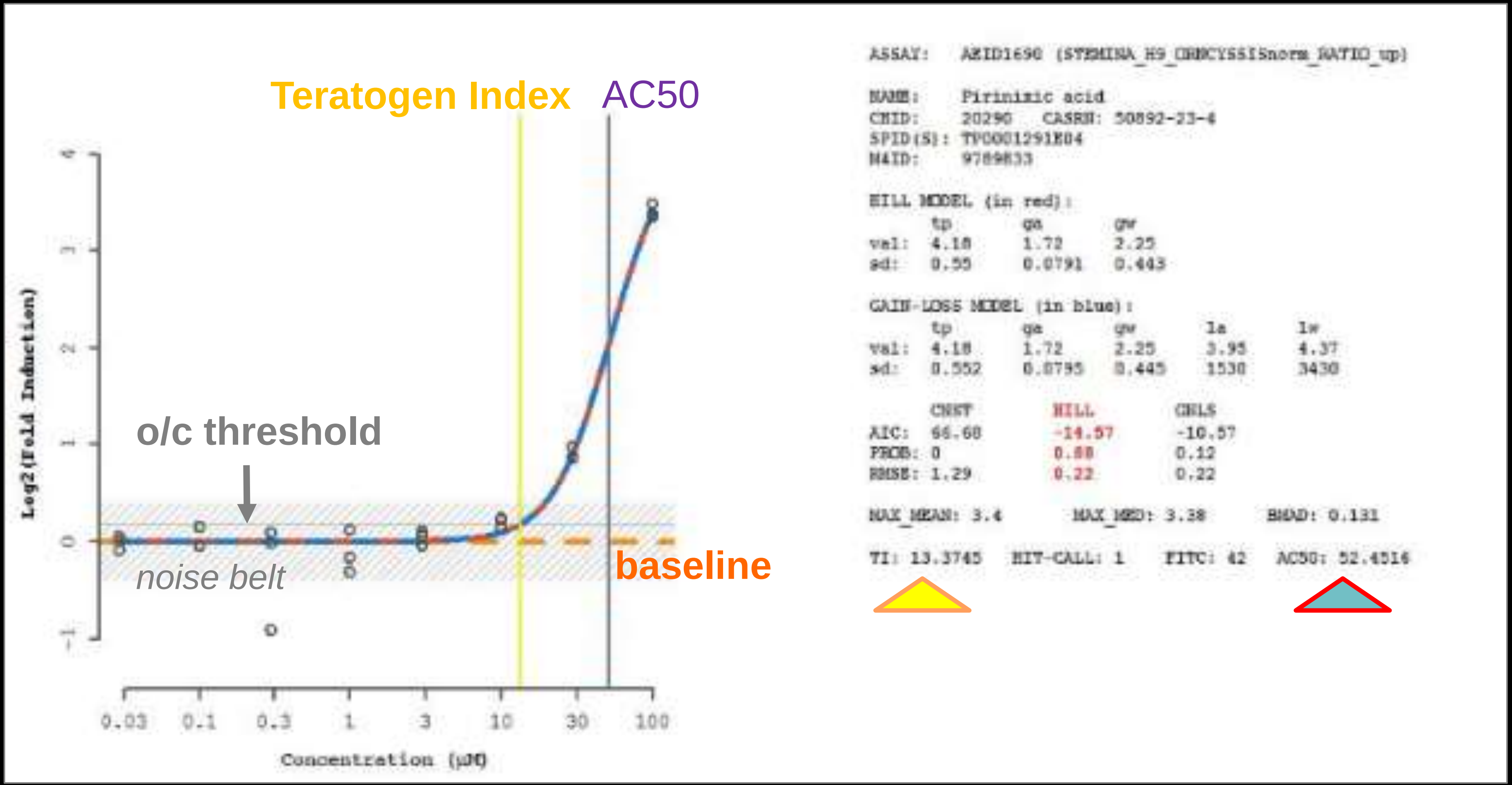
Cell viability
(normalized to control)

Teratogenicity Threshold
(o/c ratio ≤ 0.88) *

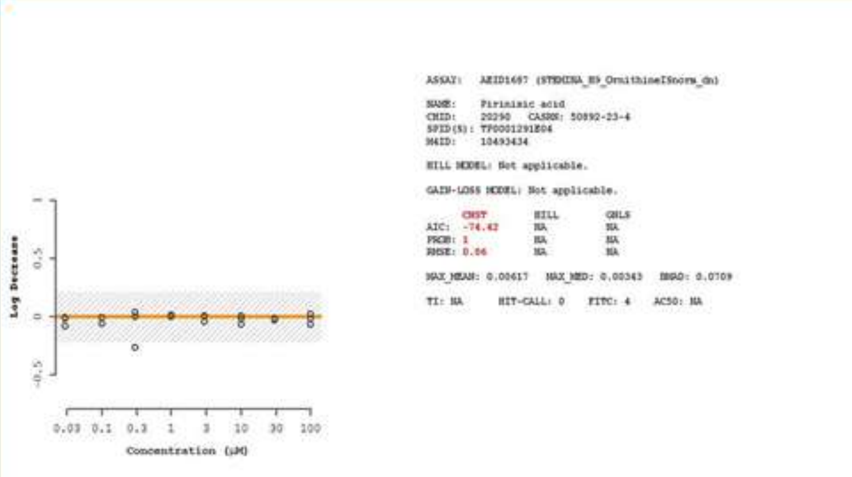
Targeted Biomarker
(o/c ratio)

** predictive model trained with 23 pharma compounds (96% accurate) and tested with 13 pharma compounds (77% accurate) [Palmer et al. 2013].*

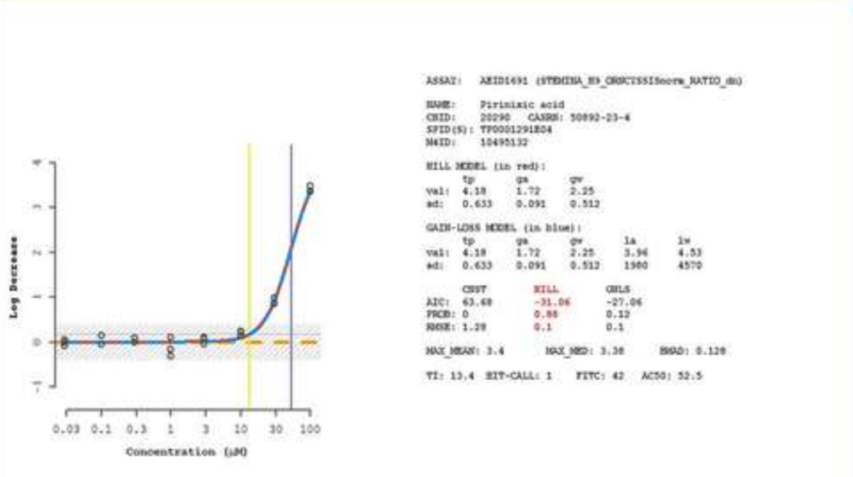
How it looks in tcpl (Level 6)



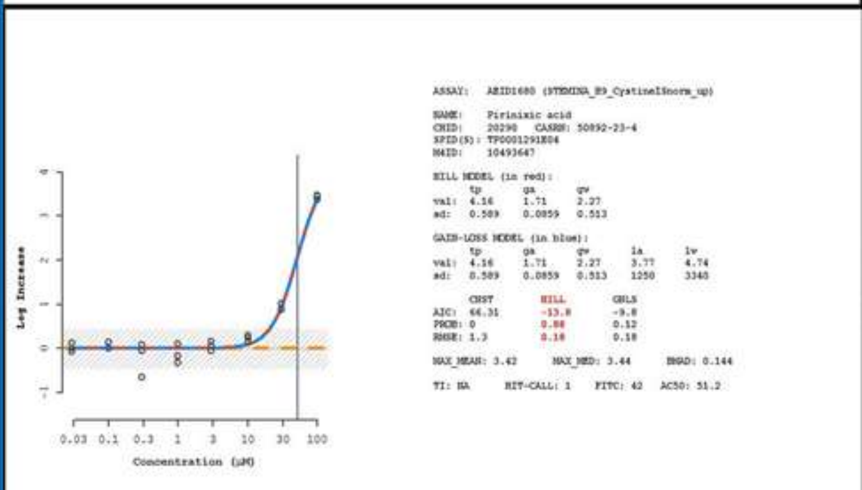
tcpl Data Representation



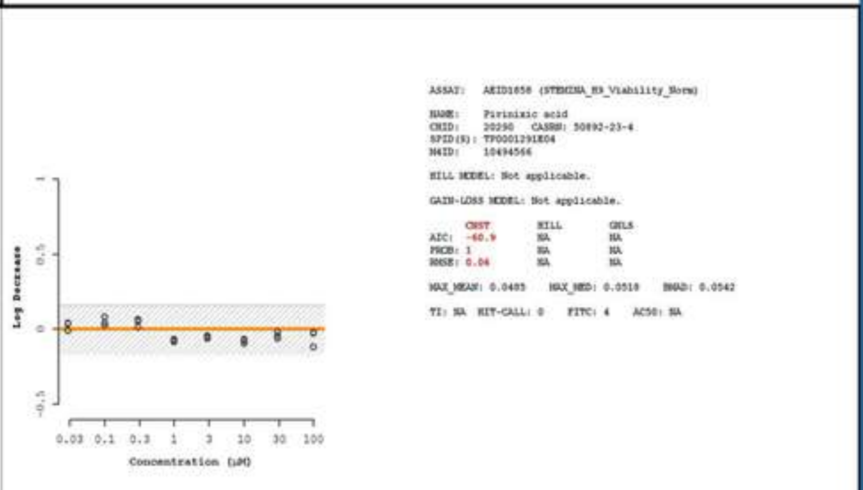
STM_ORN_down



STM_ORN/CYSS



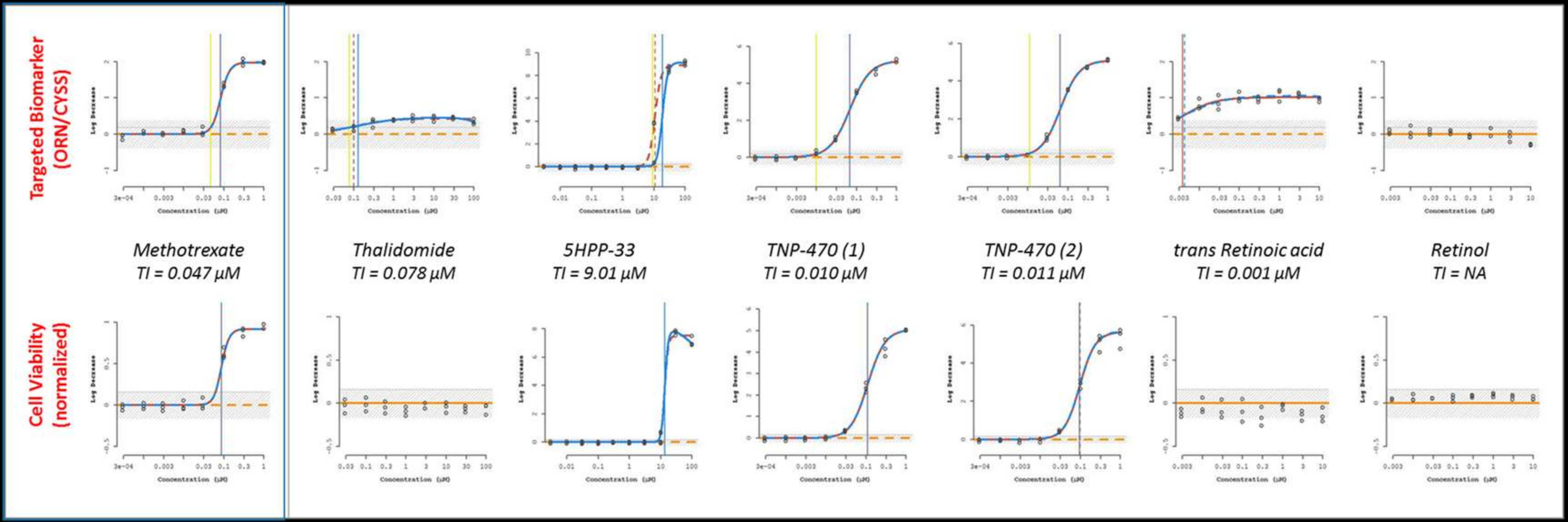
STM_CYSS_up



STM_CellViability

Examples

- Targeted biomarker sometimes co-occurs with viability, and other times not.



Results

- **177 actives (16.4%):** 172 where o/c is below CV50 and 60 without any effect on cell viability.
- **Daston List:** 10 of 28 exposure-based calls had concordance 85.7%, with caffeine and ethylene glycol failing to give the positive signal.

Chemical Name	uM dTP
trans-Retinoic acid	0.000048
PharmaGSID_47333	0.000748
3'-Azido-3'-deoxythymidine	0.047045
Thalidomide	0.078348
Mirex	0.117320
Aplaviroc hydrochloride	0.430096
Spiroxamine	0.533445
Cyclanilide	0.579090
SAR150640	0.644029
Rifampicin	1.105449
7,12-Dimethylbenz(a)anthracene	1.340670
Carbamazepine	1.421311
Etridiazole	1.465742
Tridemorph	1.561177
CP-409092	1.573462
Dihexyl phthalate	1.797381
Nitrofurazone	1.815341
Carbaryl	1.900012
AVE8488	2.367389
GW473178E methyl benzene sulphonic acid	2.382346
Darbufelone mesylate	2.511774
Besonprodil	3.024379
Diethanolamine	3.144575
Elzasonan	3.160128
Volinanserin	3.583474
PharmaGSID_47259	3.610267
Nitrilotriacetic acid	3.615839
SAR102608	4.528155
2-tert-Butyl-5-methylphenol	4.617006
Tributyl phosphate	4.779370
Carbendazim	5.380144
Lovastatin	5.826556
Cycloate	6.077669
Prometon	7.395593
N,N-Dimethyldecylamine oxide	11.451464
PharmaGSID_48507	11.939745
Pirinixic acid	13.391406
Isazofos	14.149359
Atrazine	15.622259
2-Methoxy-5-nitroaniline	15.694327
Stavudine	16.682846
Tricresyl phosphate	17.980392
2,4-Dinitrophenol	20.205362
Dinoseb	20.997202
Diallyl phthalate	21.045472
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	22.670906
Triadimenol	24.299664
Di(2-ethylhexyl) phthalate	26.003689
Procymidone	26.770940
Isopropyl triethanolamine titanate	29.417908
Clomazone	29.770828
N-Nitrosodiphenylamine	32.997148
17alpha-Hydroxyprogesterone	33.201285
Fluometuron	33.584433
Hydroxyurea	50.192887
Diuron	52.867066
Cyproconazole	61.347859
1,3-Propane sultone	69.899428
Carminic acid	84.585145
Mono(2-ethylhexyl) phthalate	123.787673

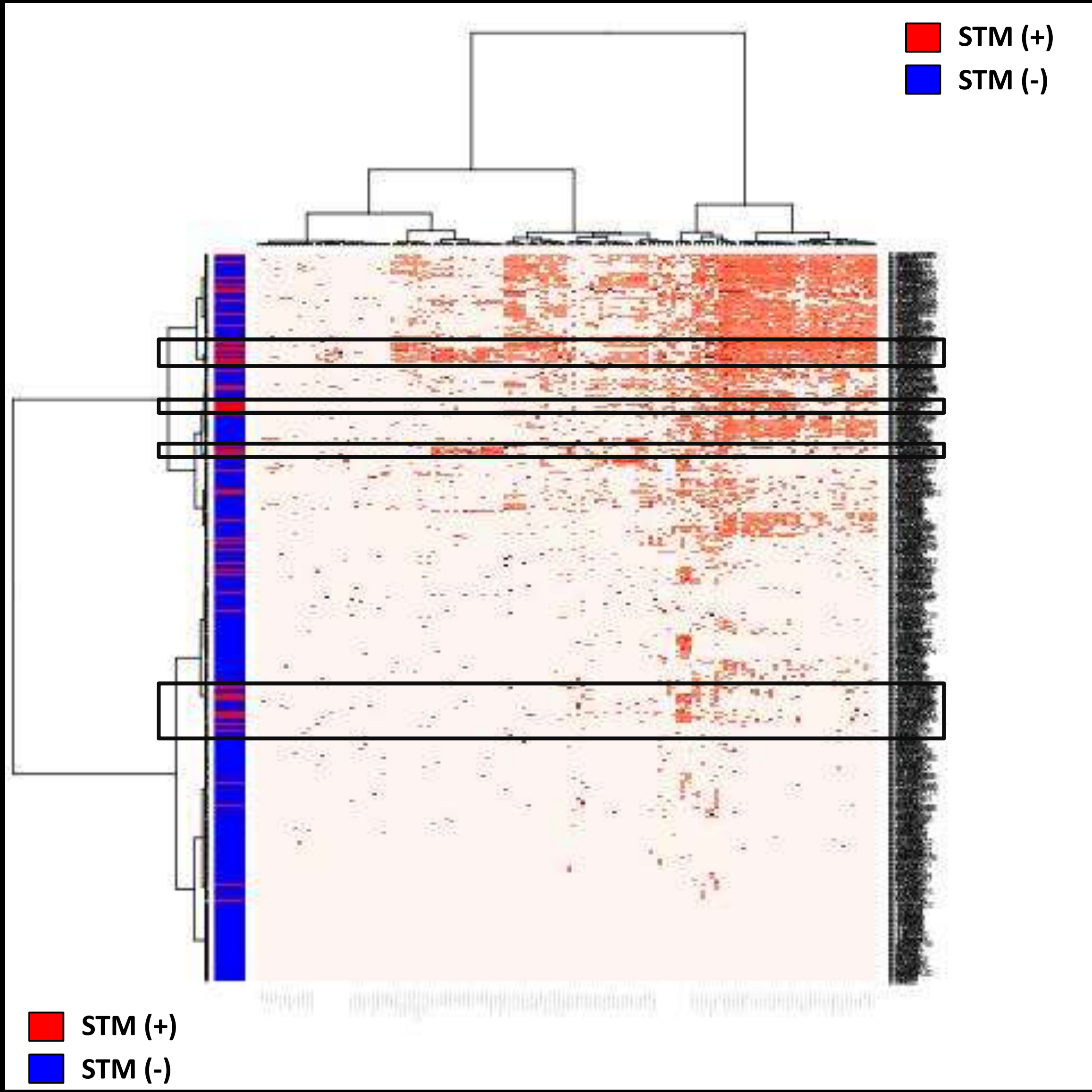
Performance Models

- **Model Performance:** range from 87-91% BA (sensitivity 0.80 to 0.86, specificity 0.93 to 1.00 depending on the anchor).
  - ECVAM/FDA labels (n=33): sens 0.80, spec 1.0, BA = 90.9%
  - add 31 literature calls (n= 64): sens 0.86, spec 0.97, BA = 92.2%
  - add 7 liberal calls (n=71): sens 0.81, spec 0.93, BA = 87.3%.

- **ToxRefDB:** sweet spot for dTP looks to be ~75 uM; preliminary model vs skeletal defects (dLEL <= 50 mg/kg).
  - 44 of 131 ToxRefDB_dev calls STM-positive
  - 812 of 948 non-calls were STM-negative
  - sensitivity (0.36), specificity (0.86) for BA = 79.3%.

Chemical Name	uM dTP	Class
trans-Retinoic acid	0.006000	X
Cytarabine hydrochloride	0.036753	D
Methotrexate	0.046665	X
Thalidomide	0.078349	X
Diphenhydramine hydrochloride	0.387290	B
Ketoconazole	0.514342	C
Rifampicin	1.105449	C
Busulfan	1.123890	D
Carbamazepine	1.421311	C
5-Fluorouracil	1.473280	D
Amiodarone hydrochloride	3.048013	D
Lovastatin	5.826556	X
Dexamethasone sodium phosphate	31.821343	C
Hydroxyurea	50.192887	D
Indomethacin	64.572031	D
Valproic acid	112.875459	D
Salicylic acid	317.314747	C
Warfarin	1000.000000	X
Acrylamide	1000.000000	NT
Isoniazid	1000.000000	C
Dimethyl phthalate	1000.000000	NT
Folic acid	1000.000000	A
Aspirin	1000.000000	C
Acetaminophen	1000.000000	B
5,5-Diphenylhydantoin	1000.000000	D
Retinol	1000.000000	A
Caffeine	1000.000000	B
Cyclopamine	1000.000000	T
Sodium L-ascorbate	1000.000000	A
Saccharin	1000.000000	A

ToxCast – STM assay correlations



Top Hits

top 24 correlations ranked by sensitivity

biological_process	gene_target	TP	FP	FN	TN
nuclear receptor gene product	CYP2E1	52	460	21	517
oxidative stress up	NFE2L2	46	432	26	553
inflammation down	SELE	43	256	29	722
inflammation down	CD40	42	270	30	708
inflammation down	HLA-DRA	42	311	30	667
nuclear receptor Tox21 ant	THRB|THRA	42	278	31	706
nuclear receptor gene product	CYP4A11	41	294	32	683
inflammation down	SELP	40	221	32	757
nuclear receptor gene product	PEG10	40	294	33	683
nuclear receptor gene product	CYP7A1	40	331	33	646
inflammation down	CD40	39	260	33	718
nuclear receptor gene product	HMGCS2	39	272	34	705
nuclear receptor gene product	FABP1	39	315	34	662
inflammation down	CD38	38	275	34	703
nuclear receptor gene product	IGF1	38	302	35	675
inflammation down	CSF1	37	240	35	738
inflammation down	CD69	37	240	35	738
inflammation down	CSF1	37	248	35	730
androgen receptor	AR	37	238	36	746
nuclear receptor gene product	AFP	37	323	36	654
chemokine down	CCL2	36	220	36	758
chemokine down	CCL26	36	227	36	751
chemokine down	CXCL8	36	229	36	749
chemokine down	CXCL10	36	282	36	696

top 24 correlations ranked by specificity

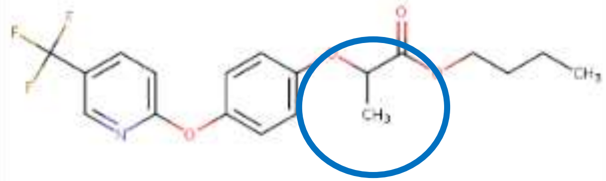
biological_process	gene_target	TP	FP	FN	TN
GPCR	Bdkrb2	5	5	67	971
GPCR	EDNRB	4	5	68	971
inflammation up	CDK2	4	7	68	969
ion channel	Grin1	4	7	68	969
GPCR	EDNRA	5	10	67	967
GPCR	NPY2R	6	12	66	964
GPCR	Grm1	6	12	66	964
nuclear receptor gene product	STAT3	5	13	68	964
GPCR	NPY	5	13	67	963
GPCR	AGTR2	5	14	67	962
nuclear receptor gene product	TGFA	4	15	69	962
nuclear receptor gene product	APOA5	4	16	69	961
transcription factor	VDR	8	17	64	968
nuclear receptor ATG	NR3C1	4	17	68	968
transcription factor	ONECUT1	4	17	68	968
GPCR	ADRB3	8	17	64	959
ppar signaling	PPARD	6	18	66	967
enzyme blocking	PDE10A	5	19	67	959
GPCR	Cckbr	5	19	67	957
GPCR	Adrb1	5	19	67	957
GPCR	DRD1	10	21	62	955
GPCR	Tacr3	6	21	66	955
cellular adhesion up	VCAM1	7	22	65	956
GPCR	Htr1a	7	22	65	954

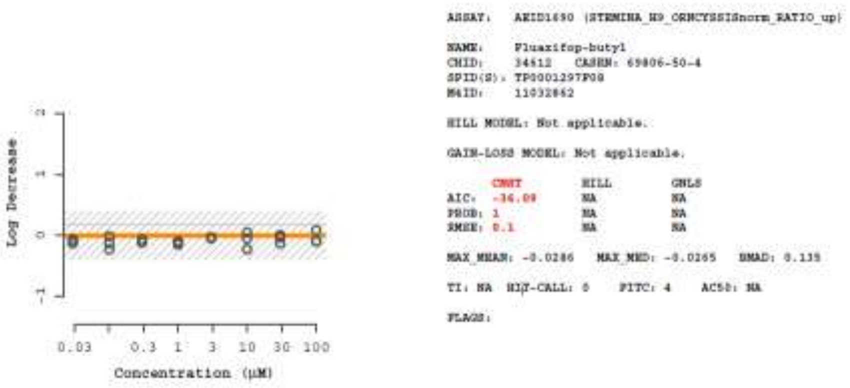
Case 1: "unknown teratogenicity" in a stereoisomer pair

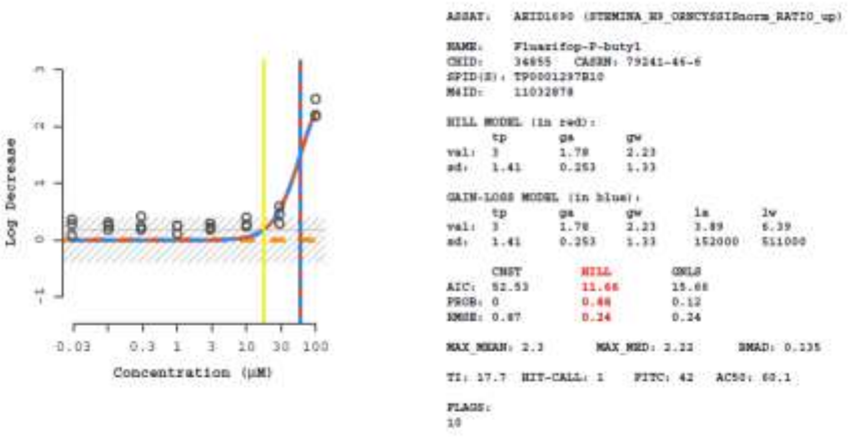
Fluazifop-butyl

69806-50-4 | DTXSID3034612

Searched by Integrated Source Name: Found 1 result for 'fluazifop butyl'.



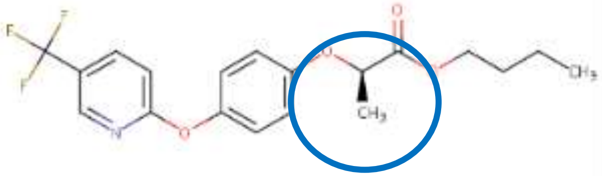




Fluazifop-P-butyl

79241-46-6 | DTXSID0034855

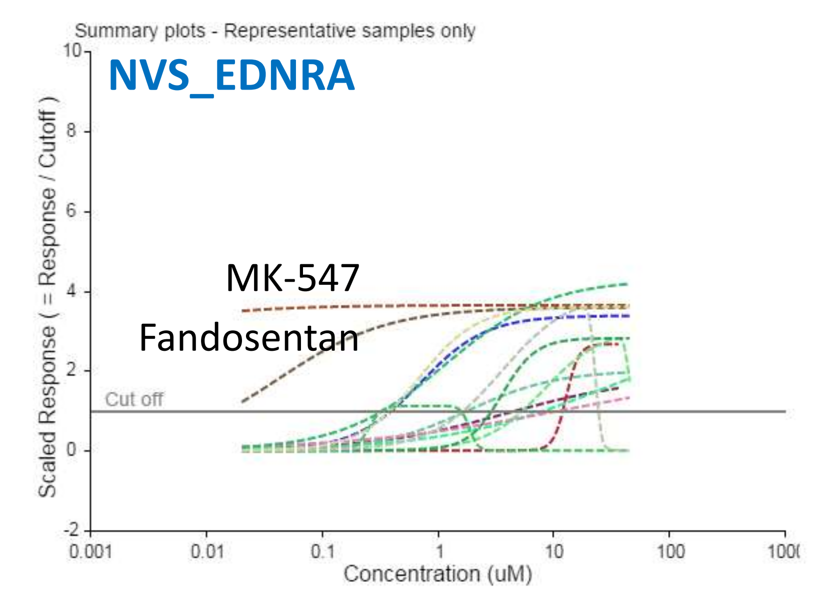
Searched by Approved Name: Found 1 result for 'fluazifop-p-butyl'.

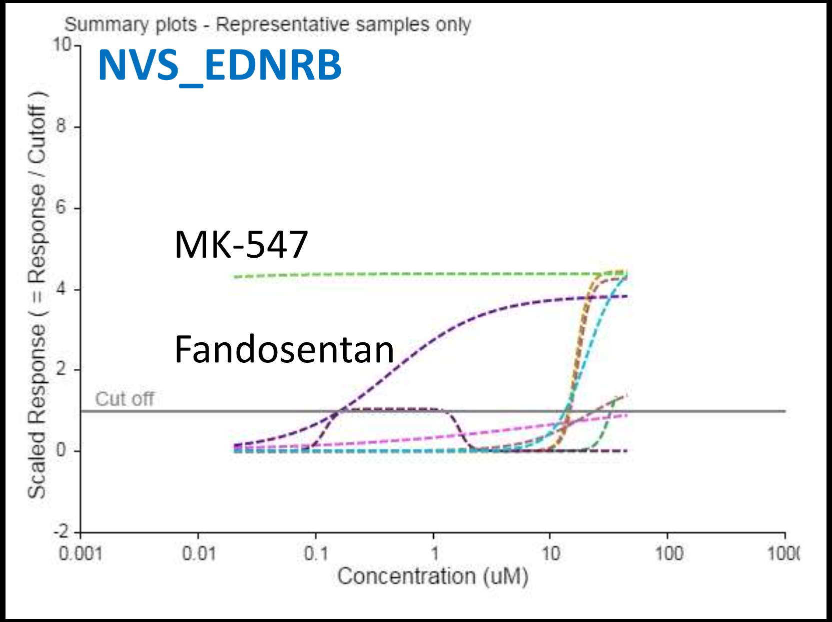


Case 2: the endothelin (ET) and endothelin receptor (EDNR) system

- ET-1/EDNRA is crucial for craniofacial/cardiac neural crest morphogenesis [Clouthier et al. 1998, Development], and ET-3/EDNRB for enteric neural crest morphogenesis [Puffenberger et al. 1994, Cell].

- Craniofacial and cardiovascular malformations were observed in rats exposed to L-753,037, a balanced EDNRA/B antagonist, similar to what is seen in knockout mice [Spence et al. 1999, Reprod Toxicol].

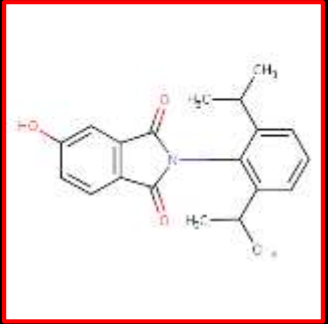




Despite their strong effects on **EDNRA** and **EDNRB** endothelin-binding assays, neither antagonist yielded a signal in the STM platform (HTC = 10 or 20 µM)

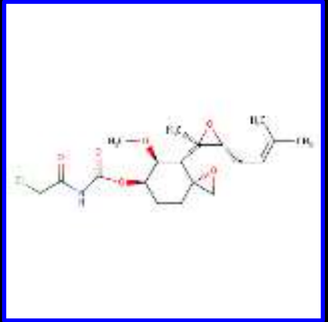
Case 3: potential vascular disrupters (pVDCs)

5HPP-33

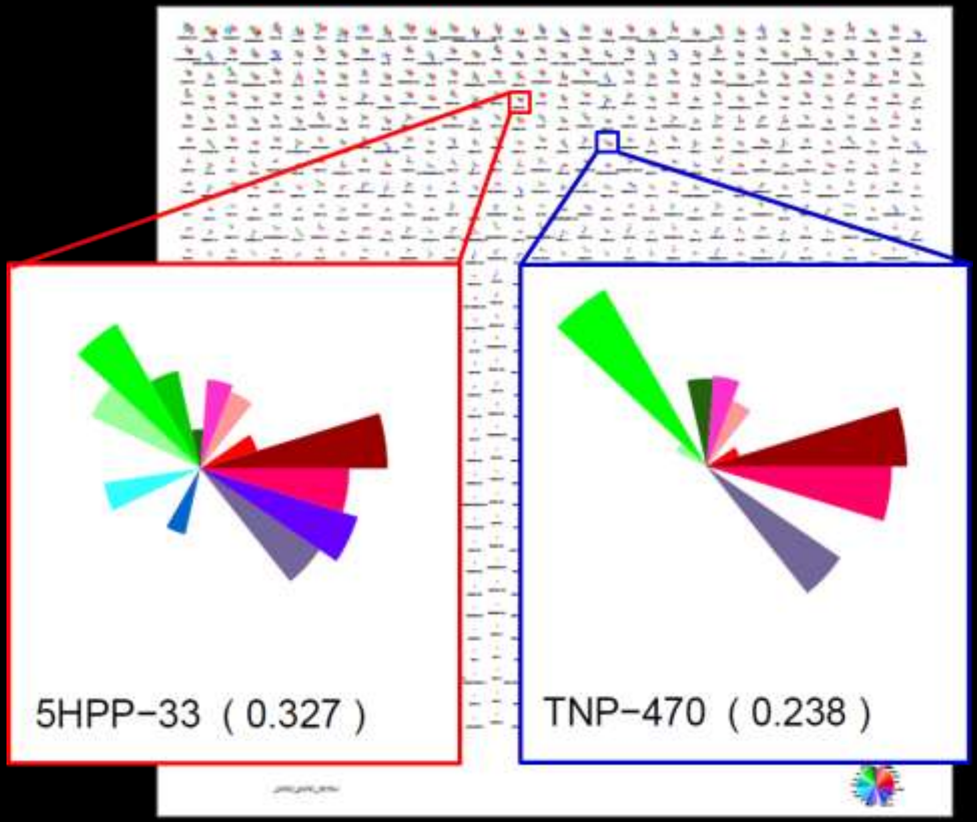


Synthetic thalidomide analog, destabilizes the tubulin network and disrupts endothelial tubulogenesis [Noguchi et al. 2005].

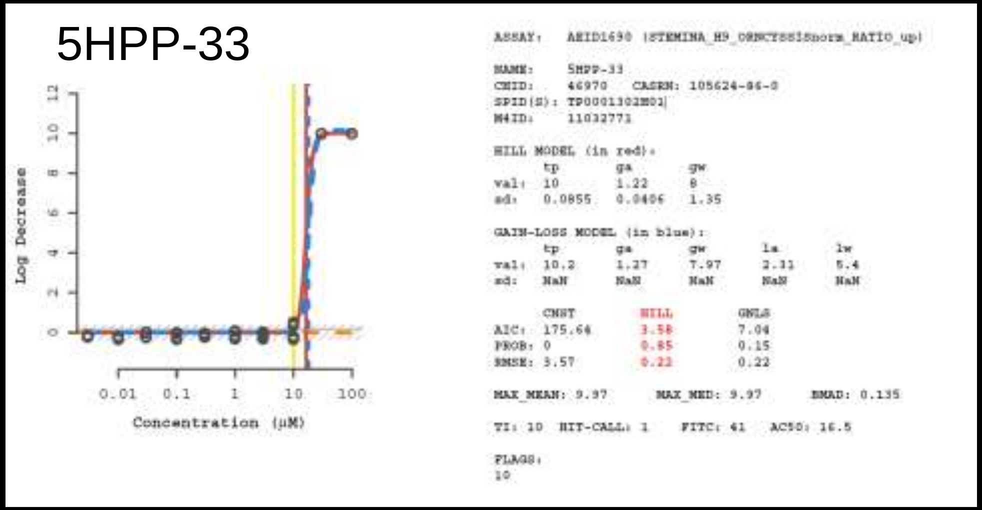
TNP-470

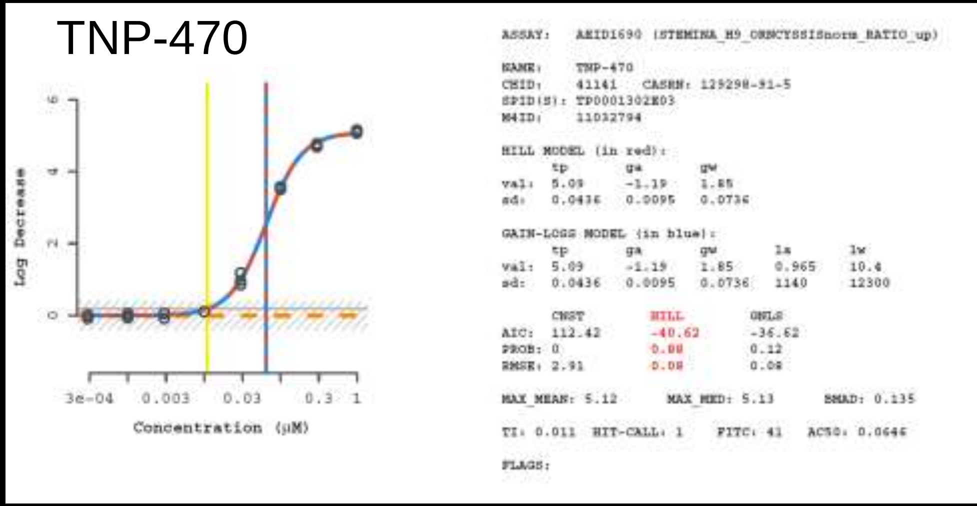


Synthetic fumagillin analog, inhibits MetAP2 and disrupts endothelial proliferation in response to Wnt signals [Griffith et al. 1998].

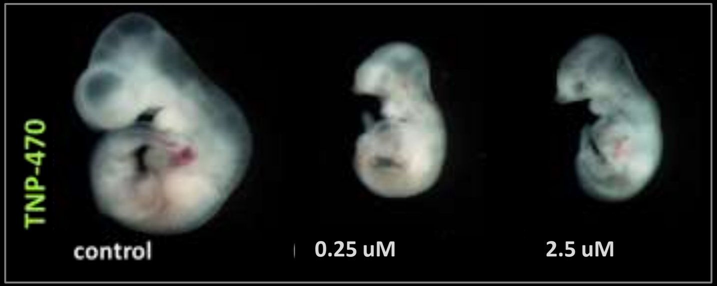


ToxPi-pVDC rank

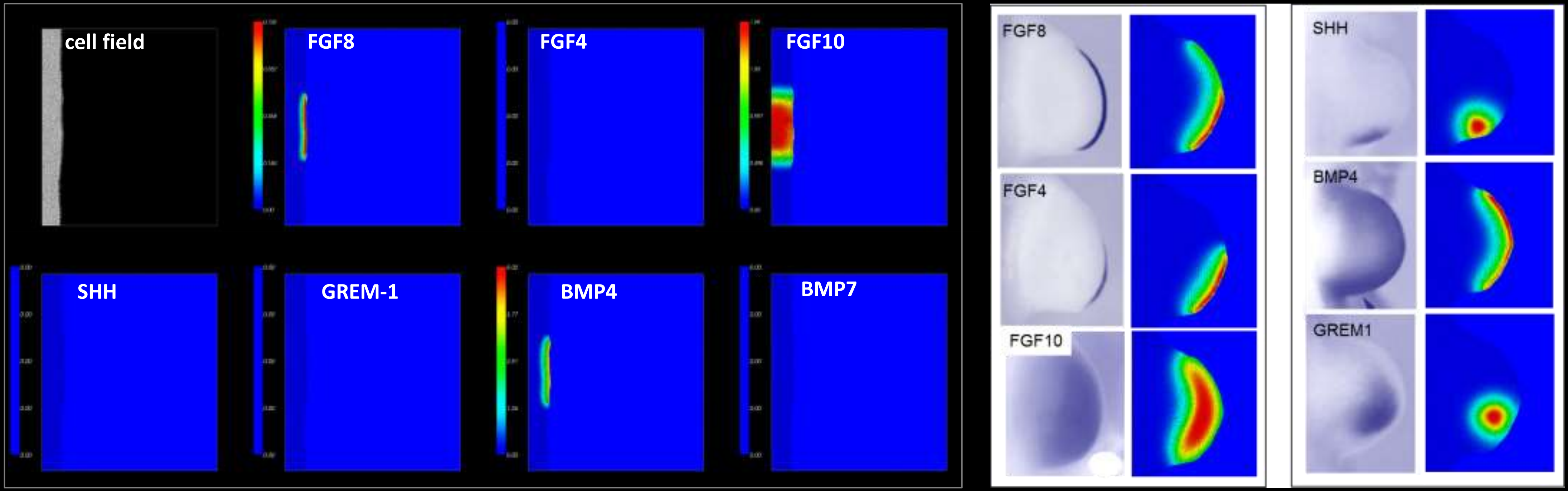




ASSAY	READOUT (uM)	HPP-33	TNP-470
FICAM tubulogenesis	AC50	0.67	2.2
Rat AEA	AC50	1.3	0.018
ArunA hNP migration/prolif	AC20	1.7	---
Tox21 p53 induction	AC50	2.6	>17.4
ZFISH embryotox (DOW)	AC50	3.4	0.032
BSK BioMAP	mTOR inhibition	4.4	0.15
STM viable cells	50% loss	7.1	5.2
STM targeted biomarker	<0.88 ORN/CYSS	9.5	0.01
VALA endothelial migration	nuCTNB	10.0	---
VALA tubulogenesis	inhibition	16.7	---
ToxCast TCB	median AC50	16.7	2.4
Rat WEC quality	AC50	21.2	0.038

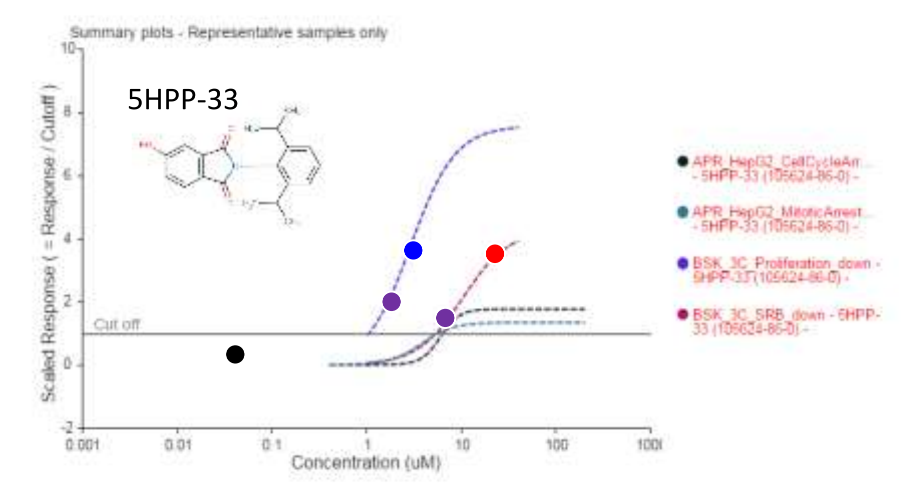


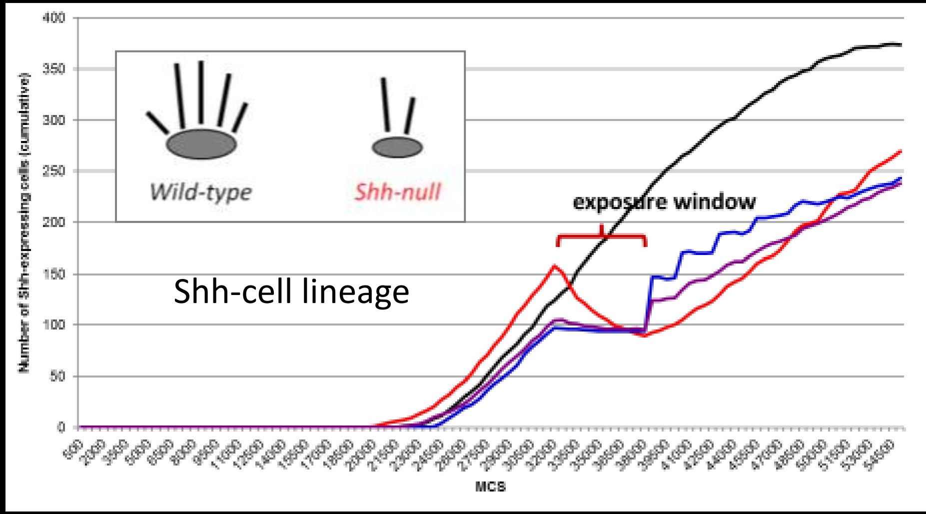
Limb-bud Outgrowth

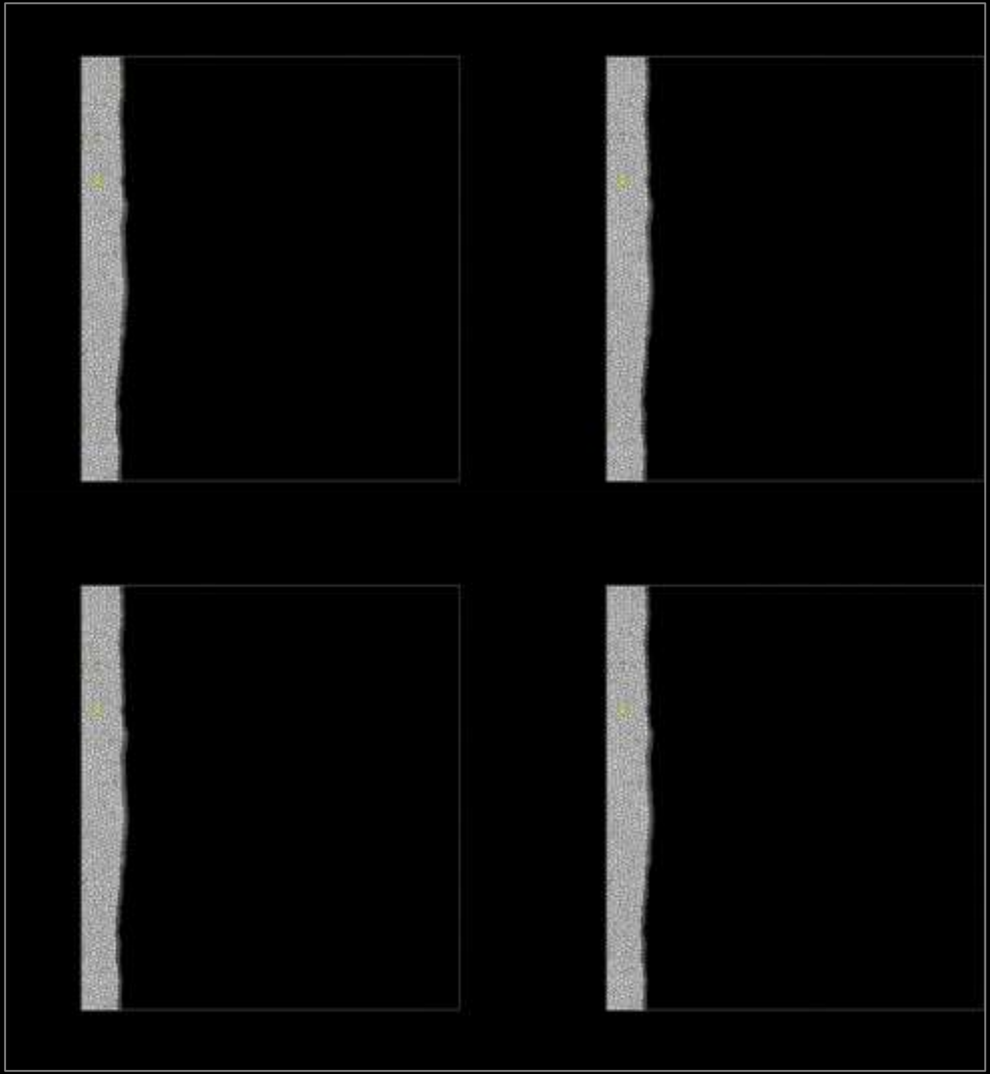


What impact would chemical disruption of cell growth and viability have?

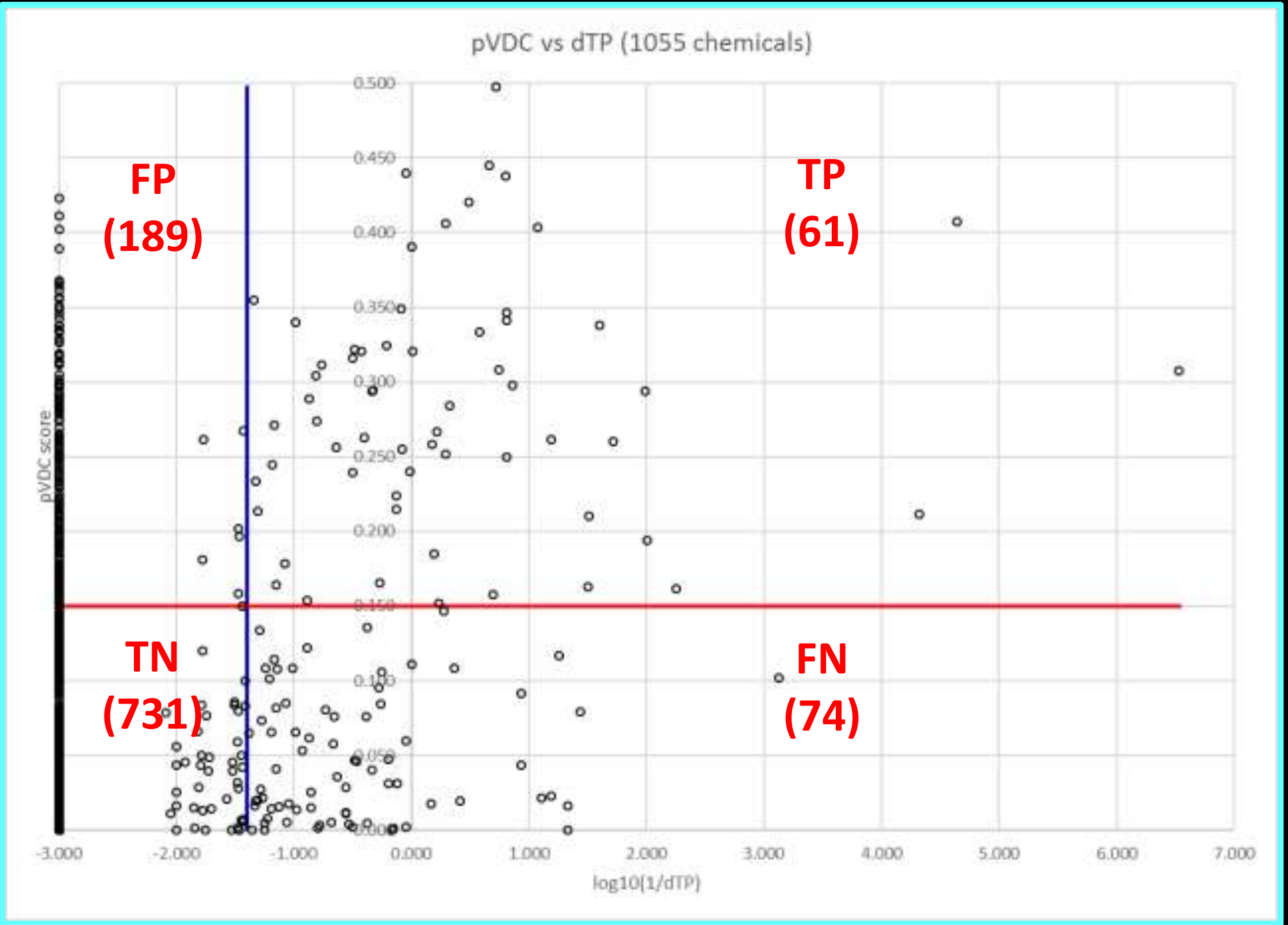
Teratogenesis *in silico*







How well does pVDC score match STM predicted teratogenicity overall?



AOP-based **pVDC** score vs **DevTox** potential from the STM hES cell platform

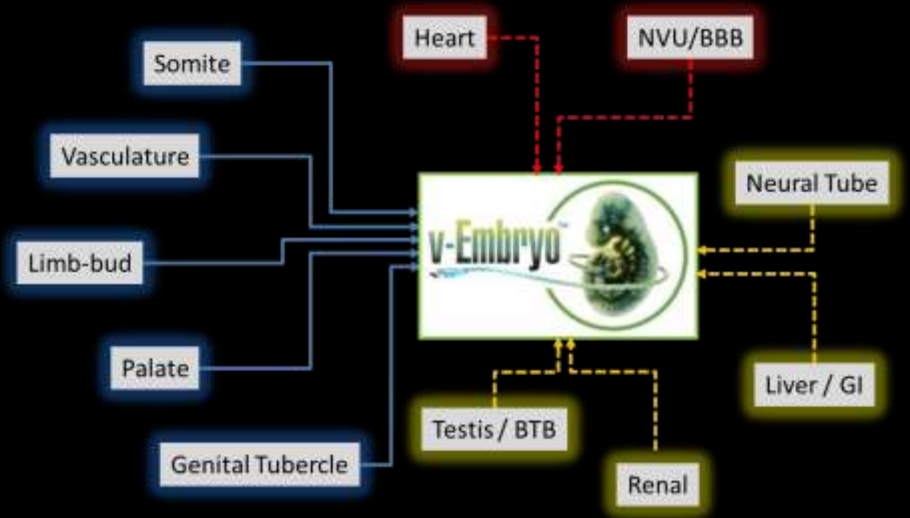
Balanced Accuracy = 75.1%
(modeled on a 38-chem test set)

24.4% pVDC(+) also STM(+)
90.8% pVDC(-) also STM(-)

Breathing life into a 'Virtual Embryo'

- **Hypothesis:** computer models that recapitulate a morphogenetic series of events can be used analytically (to understand) and theoretically (to predict) developmental toxicity.

- **Agent-Based Modeling and Simulation (ABMS):** a heuristic approach to reconstruct tissue dynamics from the bottom-up, cell-by-cell and interaction-by-interaction.



In Silico Dynamics: Computer Simulation in a Virtual Embryo (#3117):
SOT symposium 'Quantitative Systems Toxicology for Chemical Safety Assessment' [ITS]
Thursday morning

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http://www2.epa.gov/sites/production/files/2015-08/documents/virtual_tissue_models_fact_sheet_final.pdf