EUROTOX 2018, Brussels
Symposium: “Breeding IATAs for predicting DART by fusing high-dimensional data with biological knowledge”

Integrative modeling and AOP-based simulation of developmental toxicity

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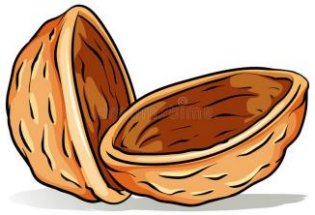
Problem statement

- Chemical exposure to a pregnant woman has the potential to affect her unborn child, leading to adverse birth outcomes and/or risks to early child development.

- Traditional animal-based methods for assessing prenatal developmental toxicity (OECD TG 414) expose pregnant rats and/or rabbits during organogenesis and necropsy at term.

- Under reauthorized TSCA (2016) the EPA must accelerate development of scientifically valid test methods to prioritize large numbers of chemicals with less reliance on animal testing.

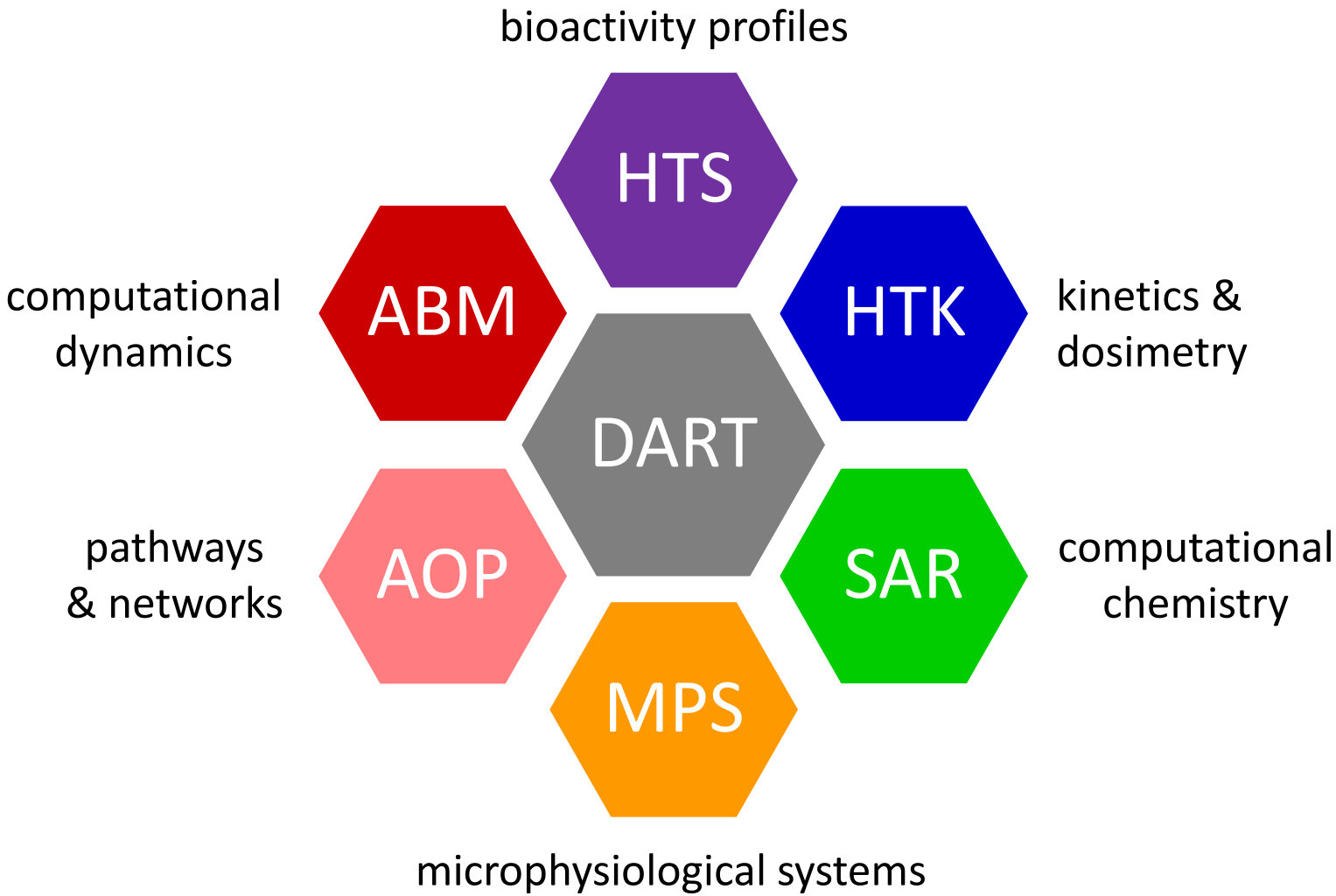
- **Challenge:** advancing actionable alternatives to vertebrate animal testing for chemical safety assessment requires *in vitro* data and *in silico* models for complex systems.



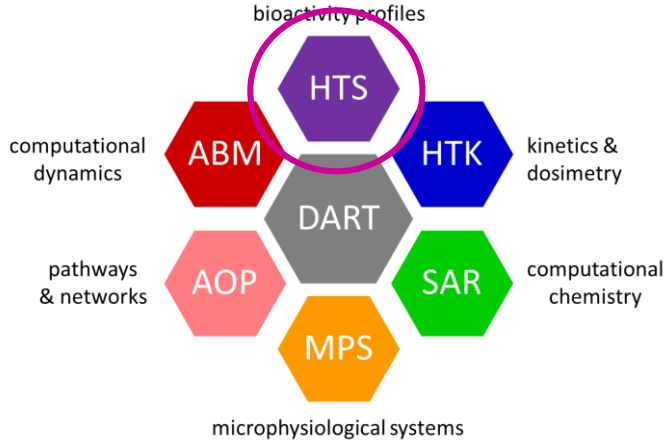
In a nutshell …

- Many alternative methods for developmental toxicity have been evaluated for 20 years; however, their application as NAMs in a regulatory setting is still poorly defined.
- IATAs can help focus DART resources by strategically targeting developmental hazards predicted by alternative *in vitro* assays and ‘non-testing’ *in silico* platforms.
- Advances in biomedical engineering and high-throughput screening enable *in vitro* profiling of chemical bioactivity for developmental processes and toxicities.
- Integration of *in vitro* data with *in silico* models that recapitulate tissue dynamics of embryonic development can reconstruct phenotypic systems synthetically.
- Computational biology is uniquely position to translate AOP-based IATAs predictively and mechanistically with regards to anatomical development.

IATA: computational synthesis and integration

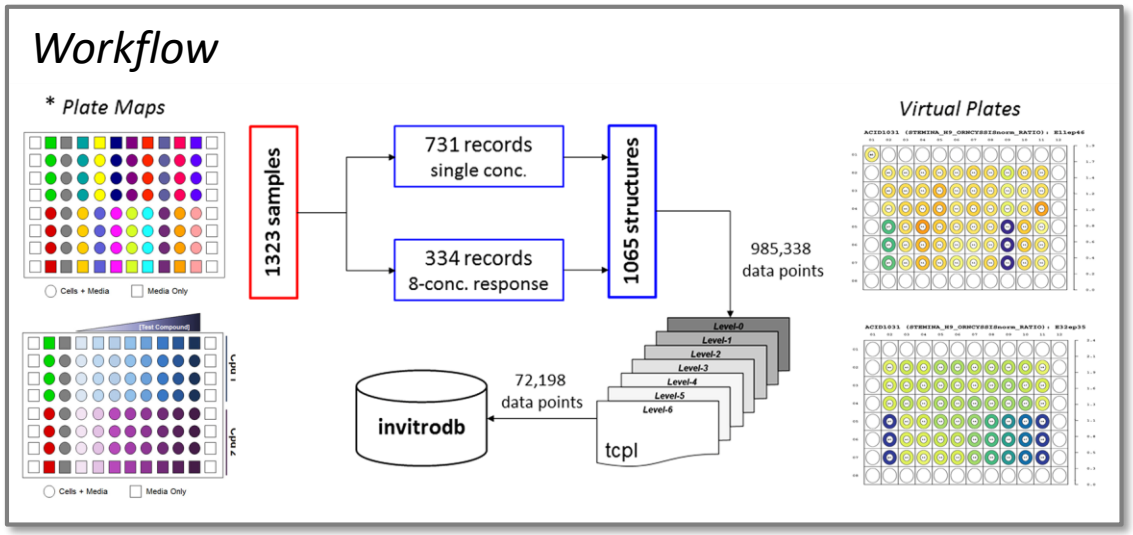


ToxCast STM platform: devTOX^{qP} assay from Stemina

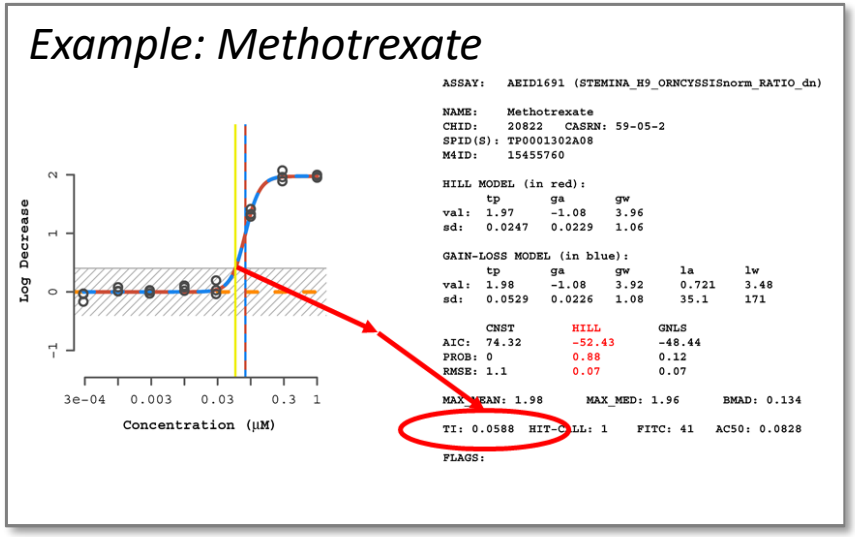


Pluripotent H9 human embryonic stem cell metabolomics assay that "… identified the potential developmental toxicants in the test set with 77% accuracy (57% sensitivity, 100% specificity)."
Palmer et al. (2013) Birth Def Res

Workflow



Example: Methotrexate



- Data processed through tcpl (Lvl 6) yielding teratogenic index (ORN:CYSS biomarker).
- Viable cell point of departure equates to 11% reduction in cell number.
- Positive response on 181 of 1065 (17%) chemicals tested to date.

Performance Check

- ToxCast_STM anchored to 42 DevTox benchmark compounds aimed at assessing alternative models[1] and having information on pregnancy risk.
- Overall accuracy of 78.6% (0.65 sensitivity, 1.00 specificity, MCC = 0.647).
- Consistent with Palmer et al. (2013) pharma-trained model 77% accuracy (0.57 sensitivity, 1.00 specificity).

How does the STM prediction do with ToxRefDB (v1) prenatal developmental toxicity studies?

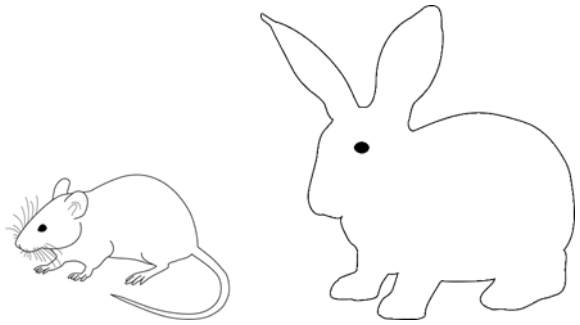
[1] Genschow et al. 2002; West et al. 2010; Daston et al. 2014; Augustine-Rauch et al. 2016; Wise et al. 2016

CASRN	Chemical	HTC[1] (µM)	CV[2] (µM)	TI[3] (µM)	Preg.class[4]	STM class[5]
302-79-4	all-trans-Retinoic acid	10	NA	0.003	X	TP
69-74-9	Cytarabine hydrochloride	1	0.083	0.054	D	TP
59-05-2	Methotrexate	1	0.062	0.059	X	TP
147-24-0	Diphenhydramine hydrochloride	100	3.76	0.588	B	TP
50-35-1	Thalidomide	100	NA	1.27	X	TP
51-21-8	5-Fluorouracil	1	1.45	2.02	D	TP
298-46-4	Carbamazepine	100	NA	2.29	C	TP
55-98-1	Busulfan	100	4.91	2.31	D	TP
13292-46-1	Rifampicin	10	NA	2.46	C	TP
19774-82-4	Amiodarone hydrochloride	10	NA	5.1	D	TP
75330-75-5	Lovastatin	20	NA	5.1	X	TP
3056-17-5	Stavudine	100	NA	32.5	C	TP
2392-39-4	Dexamethasone sodium phosphate	100	21.8	37.7	C	TP
53-86-1	Indomethacin	100	44.1	72.7	D	TP
127-07-1	Hydroxyurea	1000	237	74.9	D	TP
127-01-1	Valproic acid	1000	271	155	D	TP
4376-20-9	MEHP	500	NA	167	D	TP
57-41-0	5,5-Diphenylhydantoin	100	NA	NA	D	FN
51-52-5	6-Propyl-2-thiouracil	100	NA	NA	D	FN
10043-35-3	Boric acid	40.7	NA	NA	NTP	FN
4449-51-8	Cyclopamine	10	NA	NA	D	FN
6055-19-2	Cyclophosphamide monohydrate	20	NA*	NA	D	FN
56-53-1	Diethylstilbestrol	10	NA	NA	X	FN
107-21-1	Ethylene glycol	100000	NA	NA	NTP	FN
57-30-7	Phenobarbitol sodium	100	NA*	NA	D	FN
81-81-2	Warfarin	100	NA	NA	X	FN
69-72-7	Salicylic acid	1000	1795	513	C	TN
103-90-2	Acetaminophen	100	NA*	NA	B	TN
79-06-1	Acrylamide	36	NA	NA	NTP	TN
50-78-2	Aspirin	100	NA*	NA	C	TN
80-05-7	Bisphenol A	100	39.4	NA	NTP	TN
94-26-8	Butylparaben	100	NA	NA	GRAS	TN
58-08-2	Caffeine	500	NA	NA	B	TN
464-49-3	D-Camphor	20	NA	NA	C	TN
131-11-3	Dimethyl phthalate	100	NA	NA	NTP	TN
59-30-3	Folic acid	100	NA	NA	A	TN
54-85-3	Isoniazid	8.8	NA*	NA	C	TN
57-55-6	1,2-Propylene glycol	1000000	246664	327552	NTP	TN
68-26-8	Retinol	10	NA	NA	A	TN
81-07-2	Saccharin	100	NA	NA	A	TN
134-03-2	Sodium L-ascorbate	20	NA*	NA	A	TN
599-79-1	Sulfasalazine	100	NA*	NA	B	TN
	True Positive Rate (sensitivity)		*0.29*	*0.65*		
	True Negative Rate (specificity)		*0.94*	*1*		
	Overall Accuracy		***55.00%***	***78.60%***	***(MCC = 0.647)***	

SOURCE: NCCT, manuscript in preparation

DevTox evidence calls: *ToxRefDB endpoint_summary download (10/16/15)*

CLEAR	dLEL ≤ 200 mg/kg/day dLEL < mLEL rat *and* rabbit
SOME	dLEL ≤ 200 mg/kg/day dLEL ≤ mLEL rat *or* rabbit
EQUIVOCAL	any dLEL dLEL > mLEL rat *or* rabbit
NO	no dLEL rat *or* rabbit



- provides No Effect Level (NEL)/Lowest Effect Level (LEL) across prenatal DevTox studies [Knudsen et al. 2009];
- each study attempts to achieve a maternal (mLEL) and fetal (dLEL) for developmental parameters observed at term.

Performance anchored to ToxRefDB

			Stringency Filter Applied to DevTox Anchor		
Condition[2]	***BM-42***[3]	***Base***[4]	***Low***[5]	***Medium***[6]	***High***[7]
TP	17	85	60	35	19
FP	0	14	37	23	9
FN	9	217	127	51	11
TN	16	116	208	176	88
n	42	432	432	285	127
sensitivity	0.654	0.281	0.321	0.407	0.633
specificity	1.000	0.892	0.849	0.884	0.907
PPV	1.000	0.859	0.619	0.603	0.679
NPV	0.640	0.348	0.621	0.775	0.889
ACC	78.6%	46.5%	62.0%	74.0%	84.3%
MCC	0.647	0.190	0.202	0.332	0.554
STM(+)	TI $\leq$ 200 µM	TI $\leq$ 1000 µM	TI $\leq$ 200 µM	TI $\leq$ 200 µM	TI $\leq$ 200 µM
DevTox anchor	benchmark reference set	any dLEL rat or rabbit	SOME evidence rat OR rabbit	CLEAR evidence rat OR rabbit	CLEAR evidence rat AND rabbit

SOURCE: NCCT, manuscript in preparation

Applicable Domain: STM results mined against NVS biochemical target profile

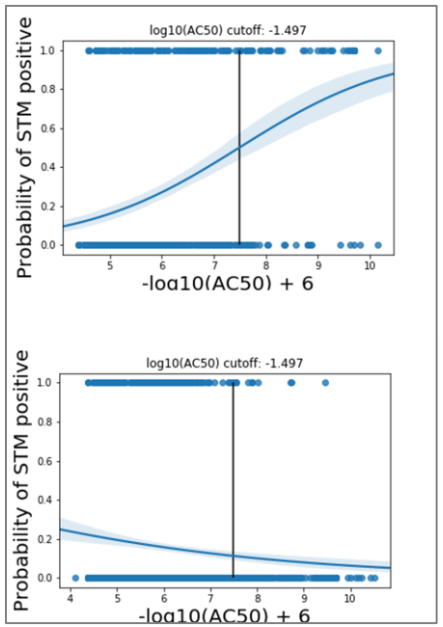
NVS ASIDs selected from invitroDB v2 (n=422)

Mine each assay for AC50 correlation against STM call (active, inactive)

Weight each target by an assay-specific logistic regression model (n=280)

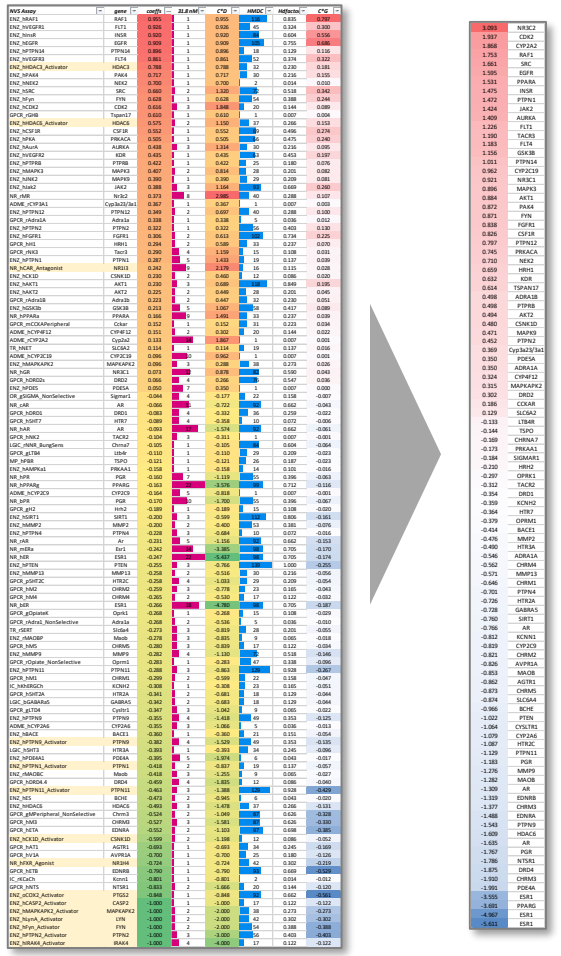
Logit model for **STM(+)** and **STM(-)** cutoff at 31.8 nM (n=116)

Human - Mouse: Disease Connection (MGI) database (n=100)



AD-plus (AC50 ≤ 31.8 nM correlate with STM(+) response

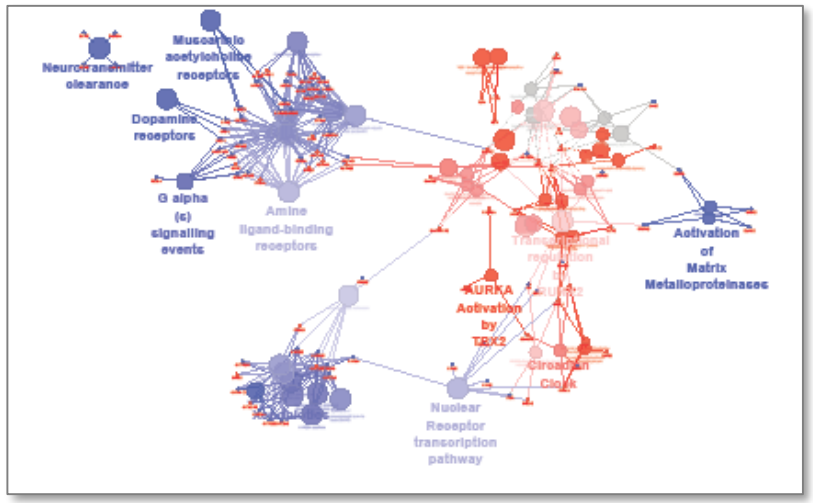
AD-minus (AC50 < 31.8 nM correlate with STM(-) response)

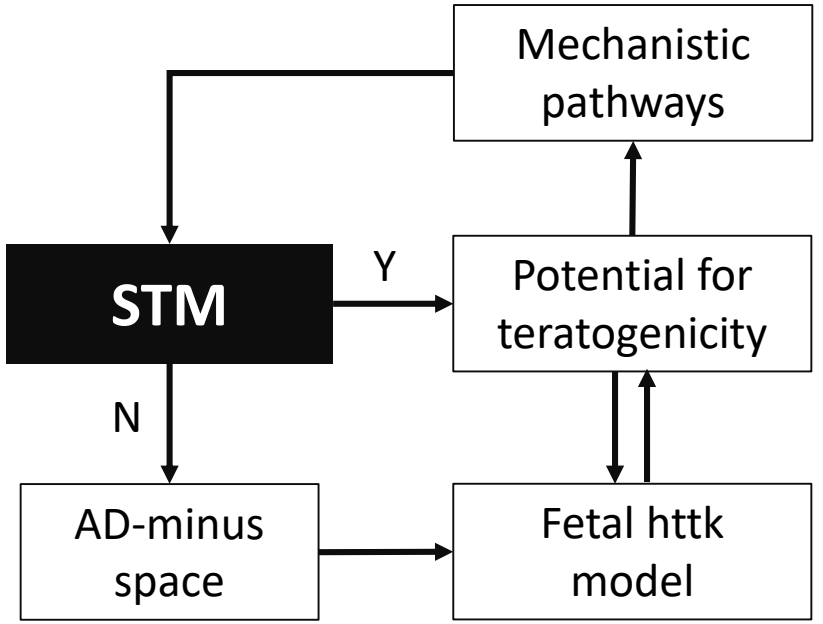


(Todd Zurlinden, NCCT)

Nuclear receptors (GR, MR, PPARA)
RTKs (VEGFR1/2/3, FGFR1, EGFR, INSR)
Kinases (RAF1, AKT1/2, SRC, FYN, PRKA, JAK2, GSK3B, MAPK3, MAPKAPK2)

3.093	NR3C2
1.937	CDK2
1.868	CYP2A2
1.753	RAF1
1.661	SRC
1.595	EGFR
1.531	PPARA
1.475	INSR
1.472	PTPN1
1.424	JAK2
1.409	AURKA
1.226	FLT1
1.190	TACR3
1.183	FLT4
1.156	GSK3B
1.011	PTPN14
0.962	CYP2C19
0.921	NR3C1
0.896	MAPK3
0.884	AKT1
0.872	PAK4
0.871	FYN
0.838	FGFR1
0.826	CSF1R
0.797	PTPN12
0.745	PRKACA
0.710	NEK2
0.659	HRH1
0.632	KDR
0.614	TSPAN17
0.498	ADRA1B
0.498	PTPRB
0.494	AKT2
0.480	CSNK1D
0.471	MAPK9
0.452	PTPN2
0.369	Cyp3a23/3a1
0.350	PDE5A
0.350	ADRA1A
0.324	CYP4F12
0.315	MAPKAPK2
0.302	DRD2



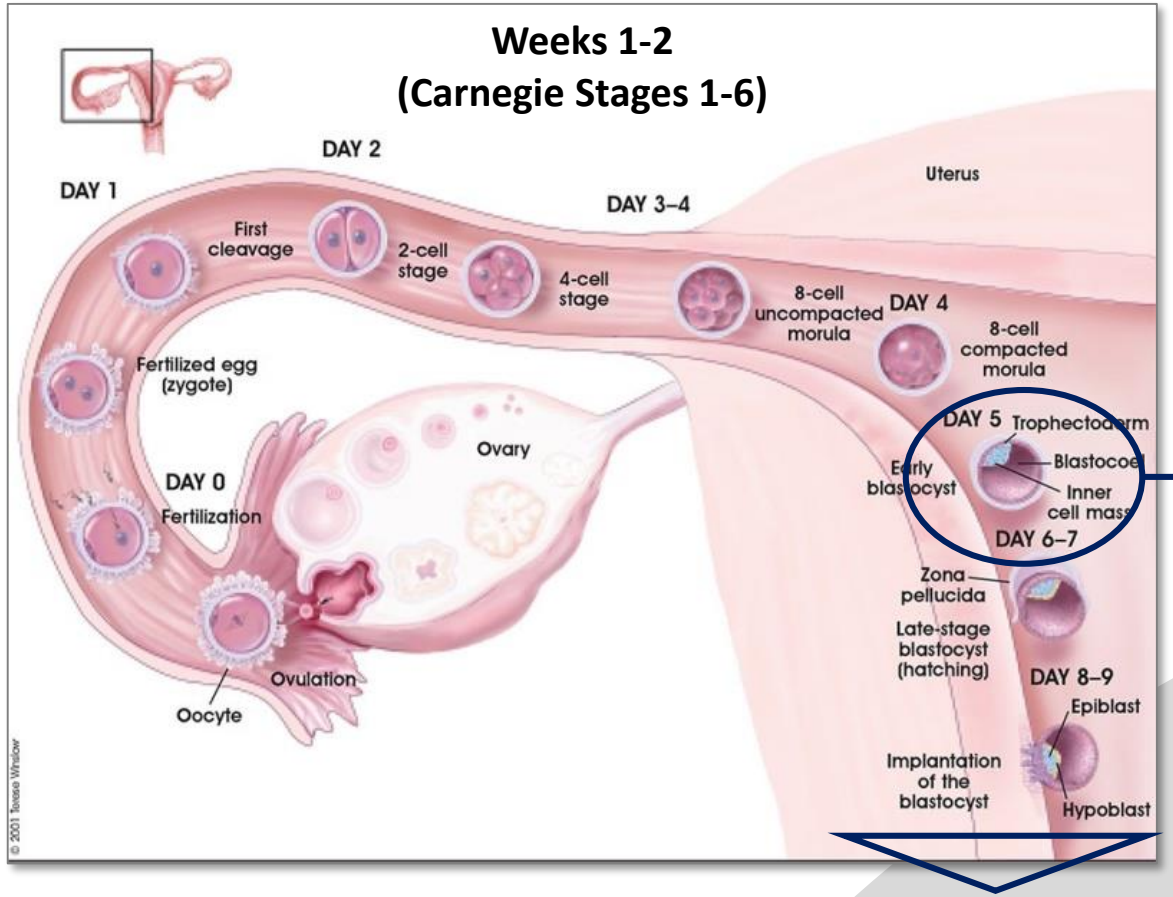


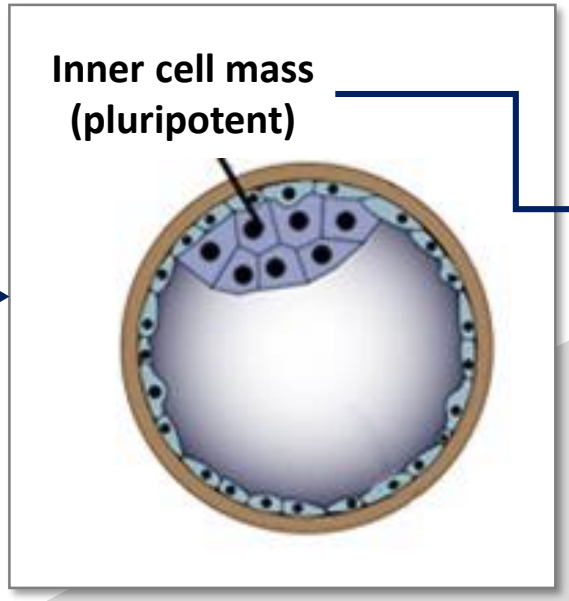
-0.562	CHRM4
-0.571	MMP13
-0.646	CHRM1
-0.701	PTPN4
-0.726	HTR2A
-0.728	GABRA5
-0.760	SIRT1
-0.766	AR
-0.812	KCNN1
-0.819	CYP2C9
-0.821	CHRM2
-0.826	AVPR1A
-0.853	MAOB
-0.862	AGTR1
-0.873	CHRM5
-0.874	SLC6A4
-0.966	BCHE
-1.022	PTEN
-1.064	CYSLTR1
-1.079	CYP2A6
-1.087	HTR2C
-1.129	PTPN11
-1.183	PGR
-1.276	MMP9
-1.282	MAOB
-1.309	AR
-1.319	EDNRB
-1.377	CHRM3
-1.488	EDNRA
-1.543	PTPN9
-1.609	HDAC6
-1.635	AR
-1.767	PGR
-1.786	NTSR1
-1.875	DRD4
-1.910	CHRM3
-1.991	PDE4A
-3.555	ESR1
-3.691	PPARG
-4.967	ESR1
-5.611	ESR1

GPCRs (CHRM1/2/3/4/5, HTR2A/2C, DRD4, EDNRA/B, …)
Nuclear receptors (ESR1, PGR, AR, PPARG)
Phosphatases (PTEN, PTPN4/9/11)

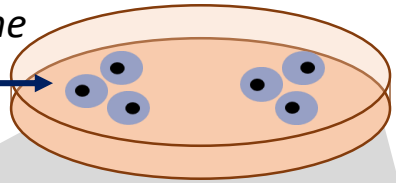
Todd Zurlinden, NCCT

TIMELINE OF HUMAN EMBRYONIC DEVELOPMENT

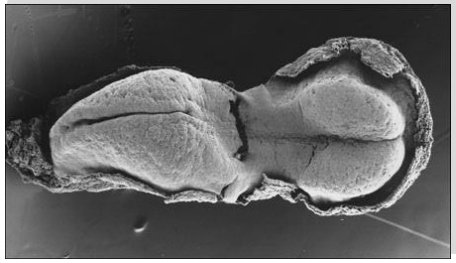




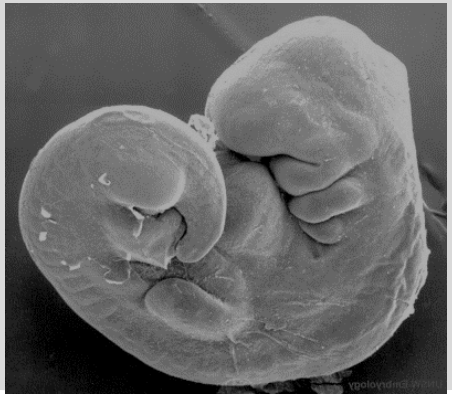
WA9 (H9)
hESC line



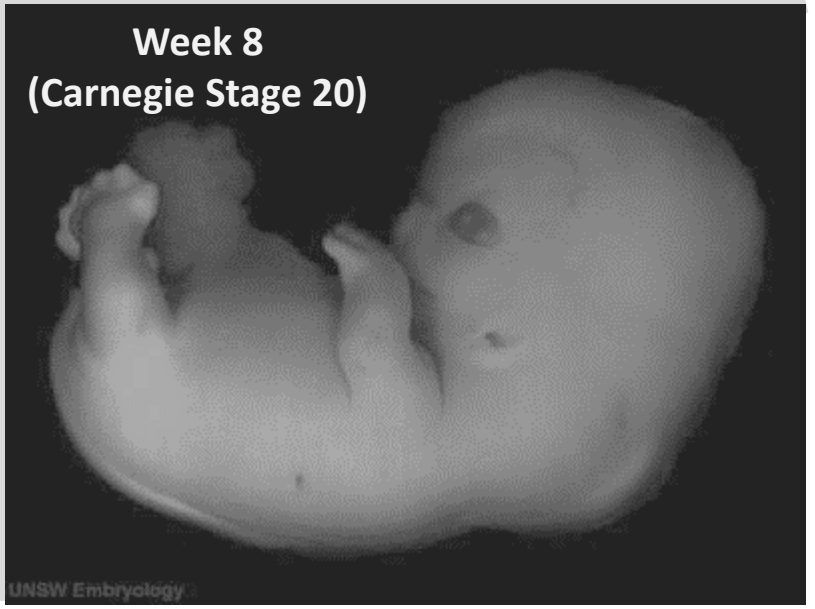
Week 3
(Carnecie Stage 8)



Week 4
(Carnecie Stage 13)

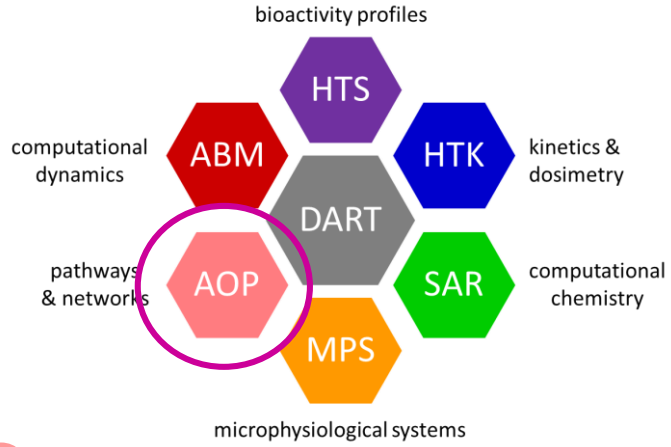


Week 8
(Carnecie Stage 20)



SOURCE: https://embryology.med.unsw.edu.au/embryology

AOP-based modeling: cleft palate as an example



ToxCast Chemicals

HTS assay results filtered-out cytotox and summarized by gene for chemi-informatics and machine-learning

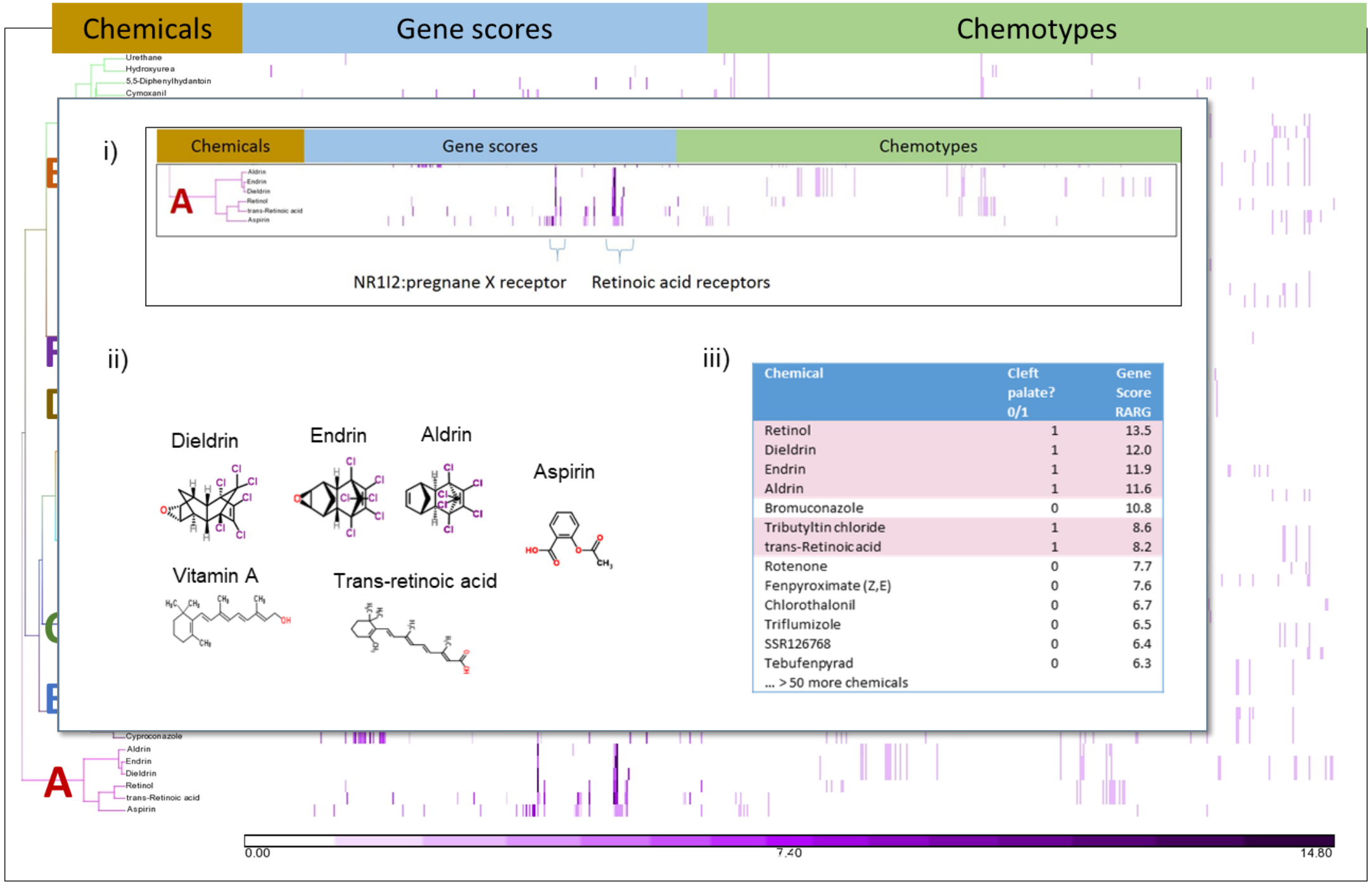
Animal studies

63 of 500 chemicals were associated with cleft palate in ToxRefDB_Dev or biomedical literature

AOP clusters

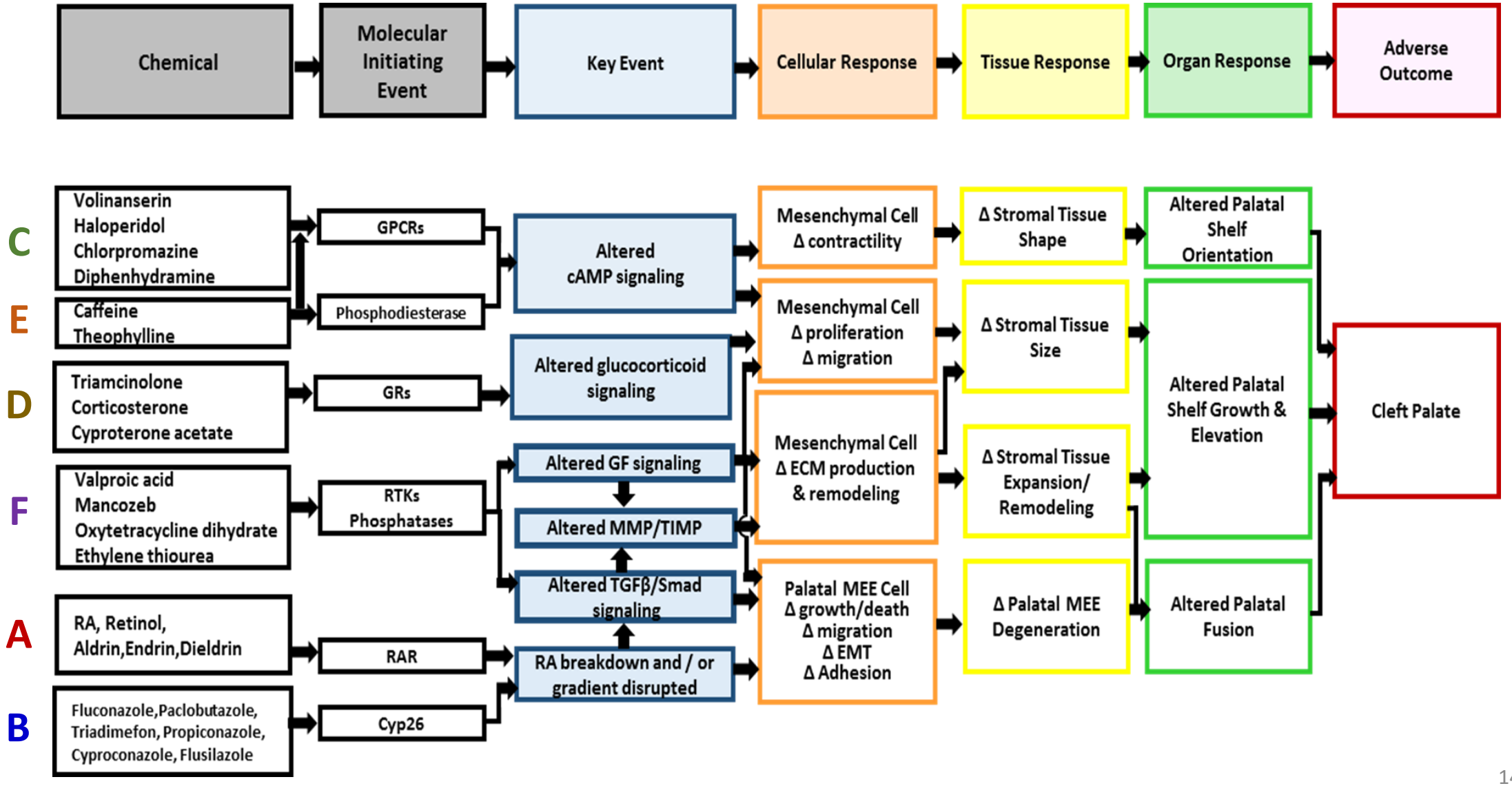
6 mechanistic profiles inferred from integration of HTS data with chemical structure.

Baker et al. (manuscript in preparation)

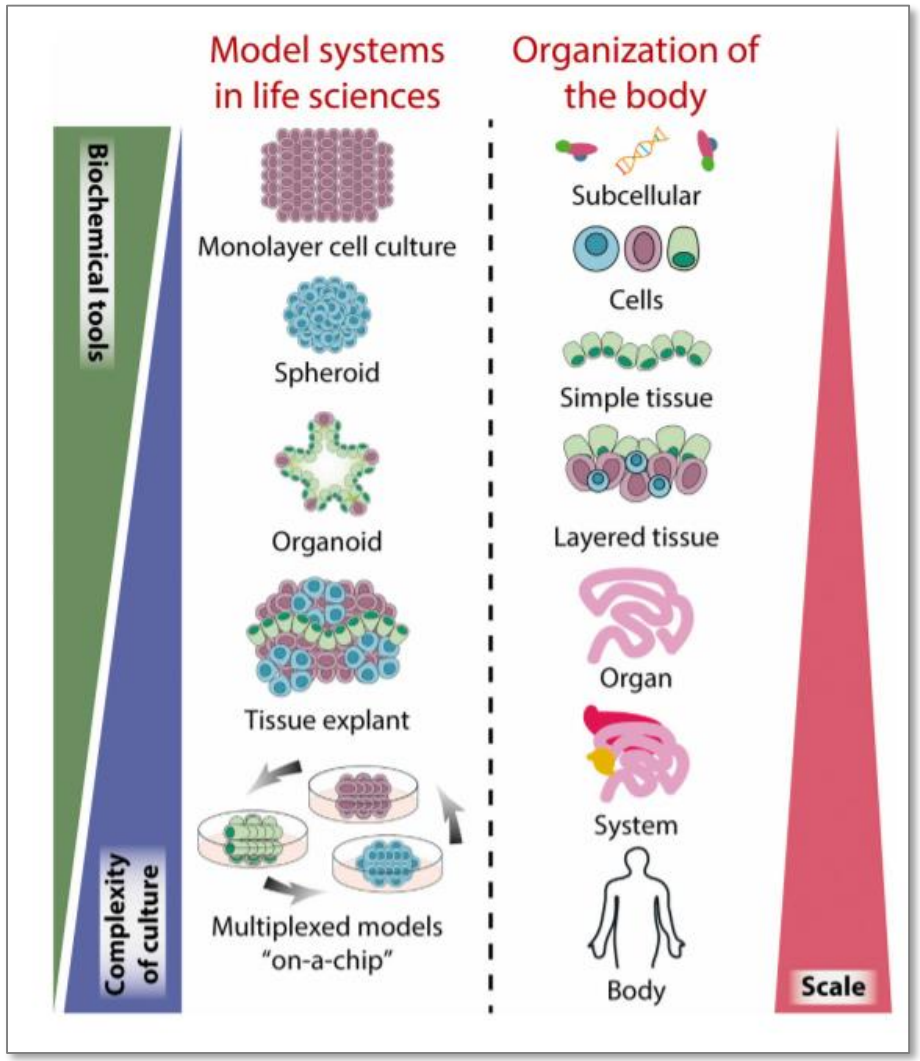


Chemical	Cleft palate? 0/1	Gene Score RARG
Retinol	1	13.5
Dieldrin	1	12.0
Endrin	1	11.9
Aldrin	1	11.6
Bromuconazole	0	10.8
Tributyltin chloride	1	8.6
trans-Retinoic acid	1	8.2
Rotenone	0	7.7
Fenpyroximate (Z,E)	0	7.6
Chlorothalonil	0	6.7
Triflumizole	0	6.5
SSR126768	0	6.4
Tebufenpyrad	0	6.3
... >50 more chemicals		

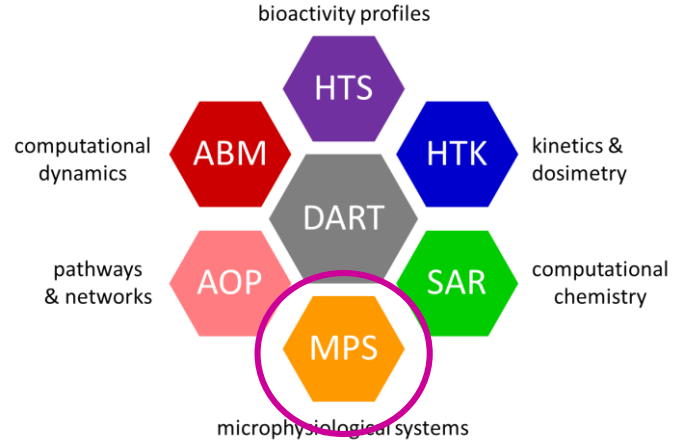
AOP clusters: inferred from chemical structure-bioactivity profiles

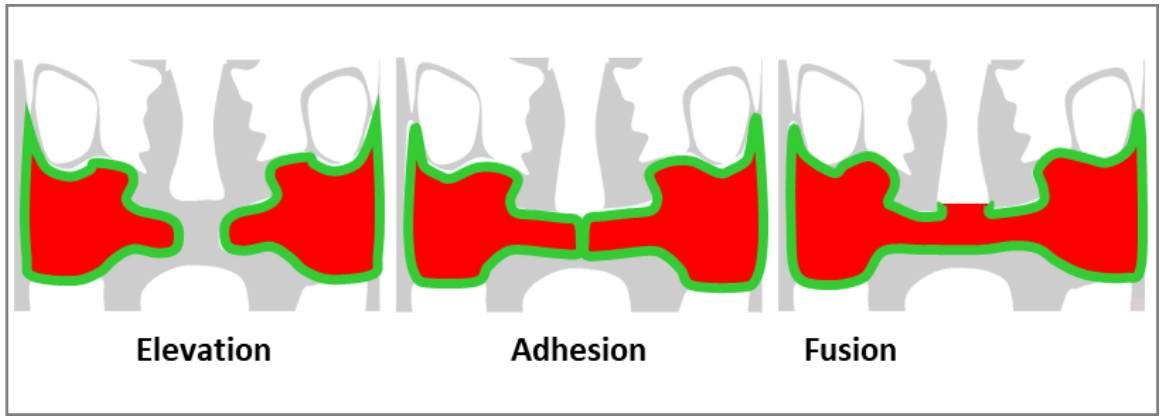


Microtissue models: reconstructing the system



SOURCE: Yin et al. Cell Stem Cell 2016.

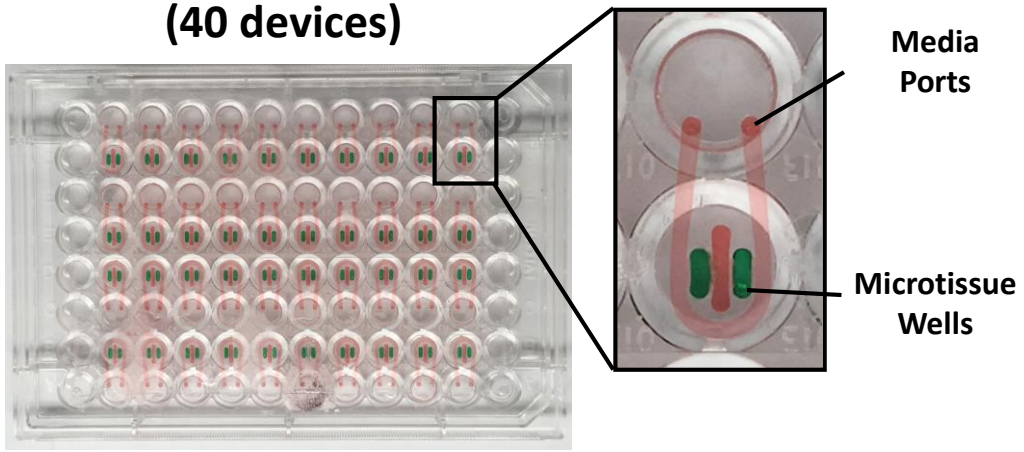


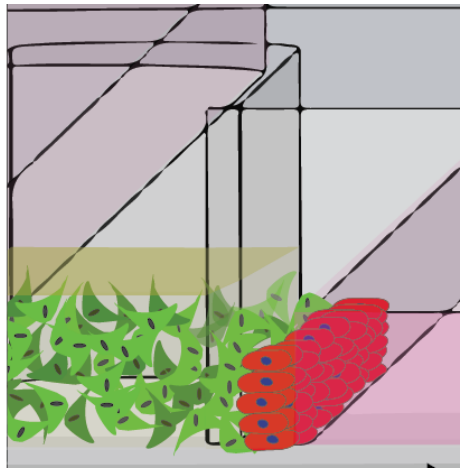


- Palatal development is structurally simple: surface epithelium covering cranial mesenchyme.
- Dynamic signaling between epithelium-mesenchyme drives outgrowth, adherence and fusion.

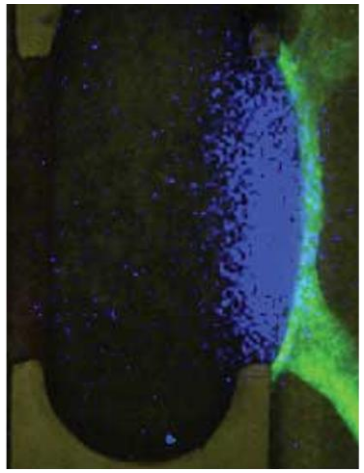
Microphysiological system: reverse-engineering E/M interactions during outgrowth

Microfluidic plates (40 devices)

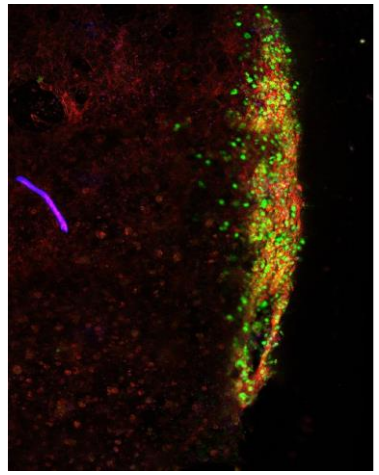




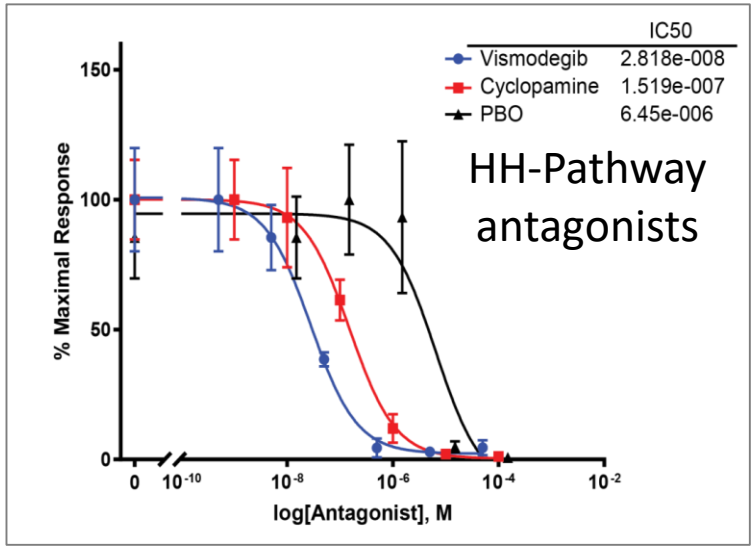
Gli-luciferase



Ki67 proliferation

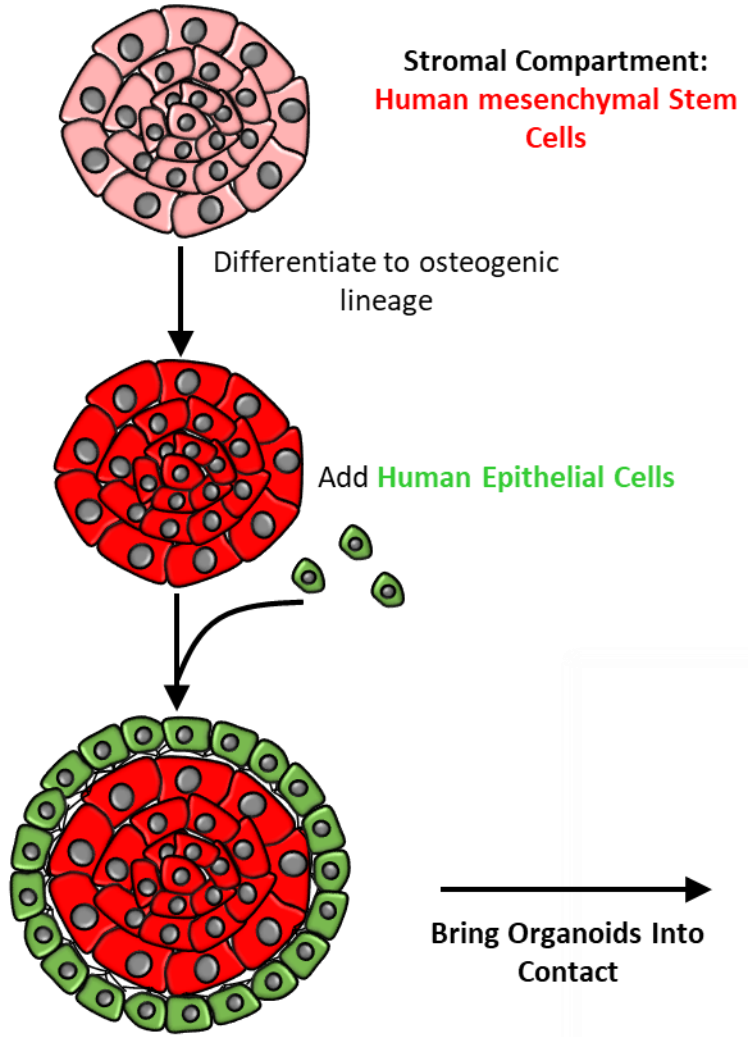


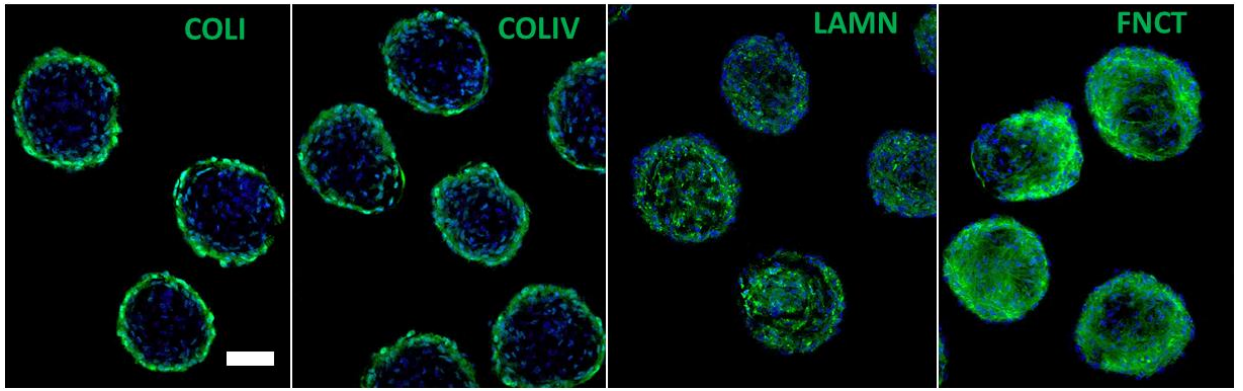
- 3D epithelial / mesenchymal organization
- directed SHH gradient and Gli1-outgrowth
- HTS and HCI amenable
- fluorescent and luminescent readouts

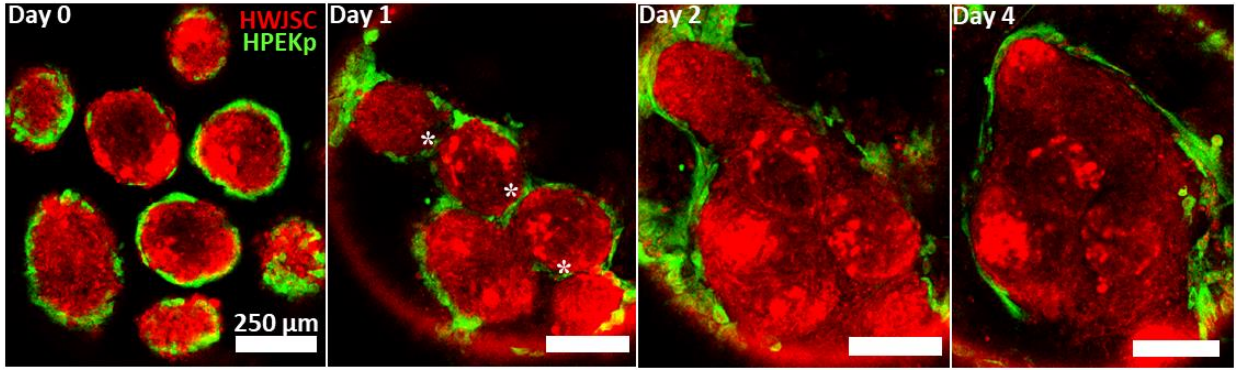


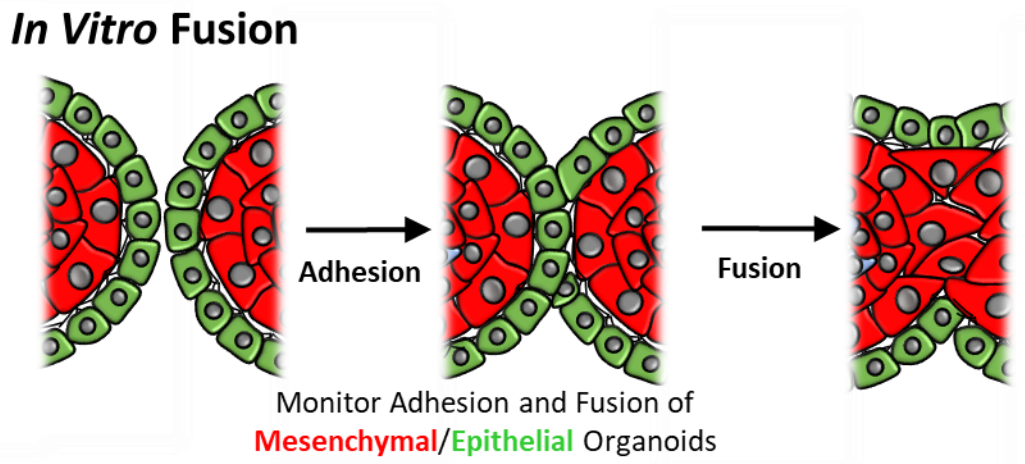
SOURCE: Brian Johnson, U Wisconsin (HMAPS)

Fusion-competent organoids



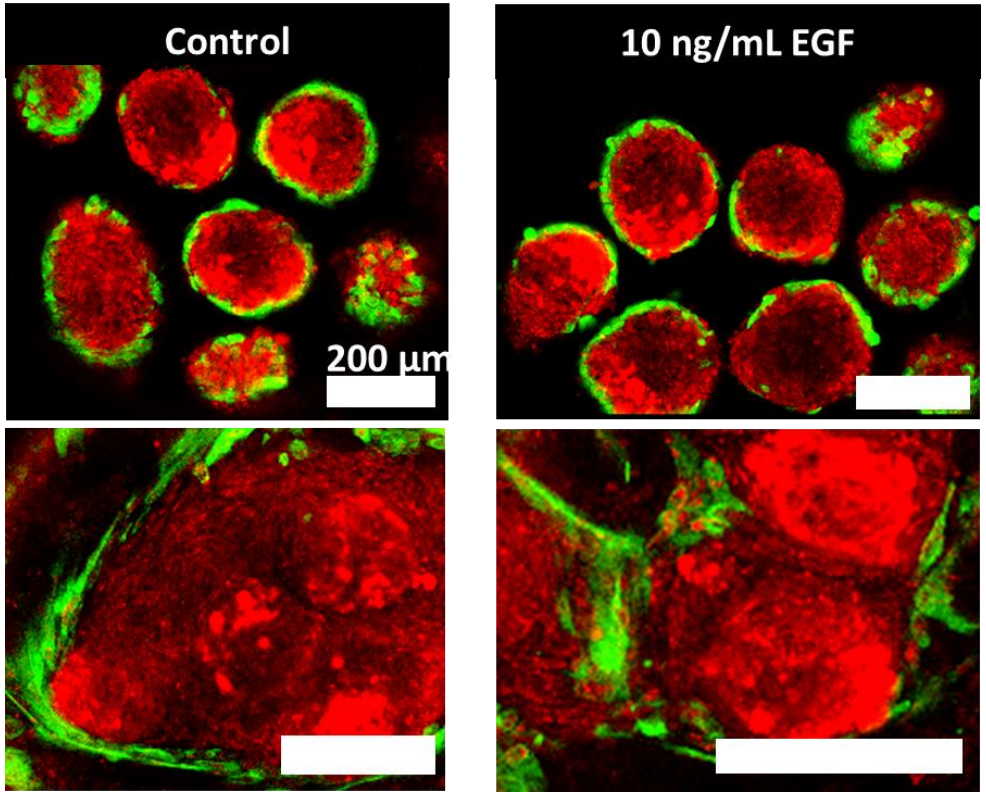






SOURCE: Belair et al. (2018) Toxicol Sci

Fusion is delayed by excessive EGF Signaling

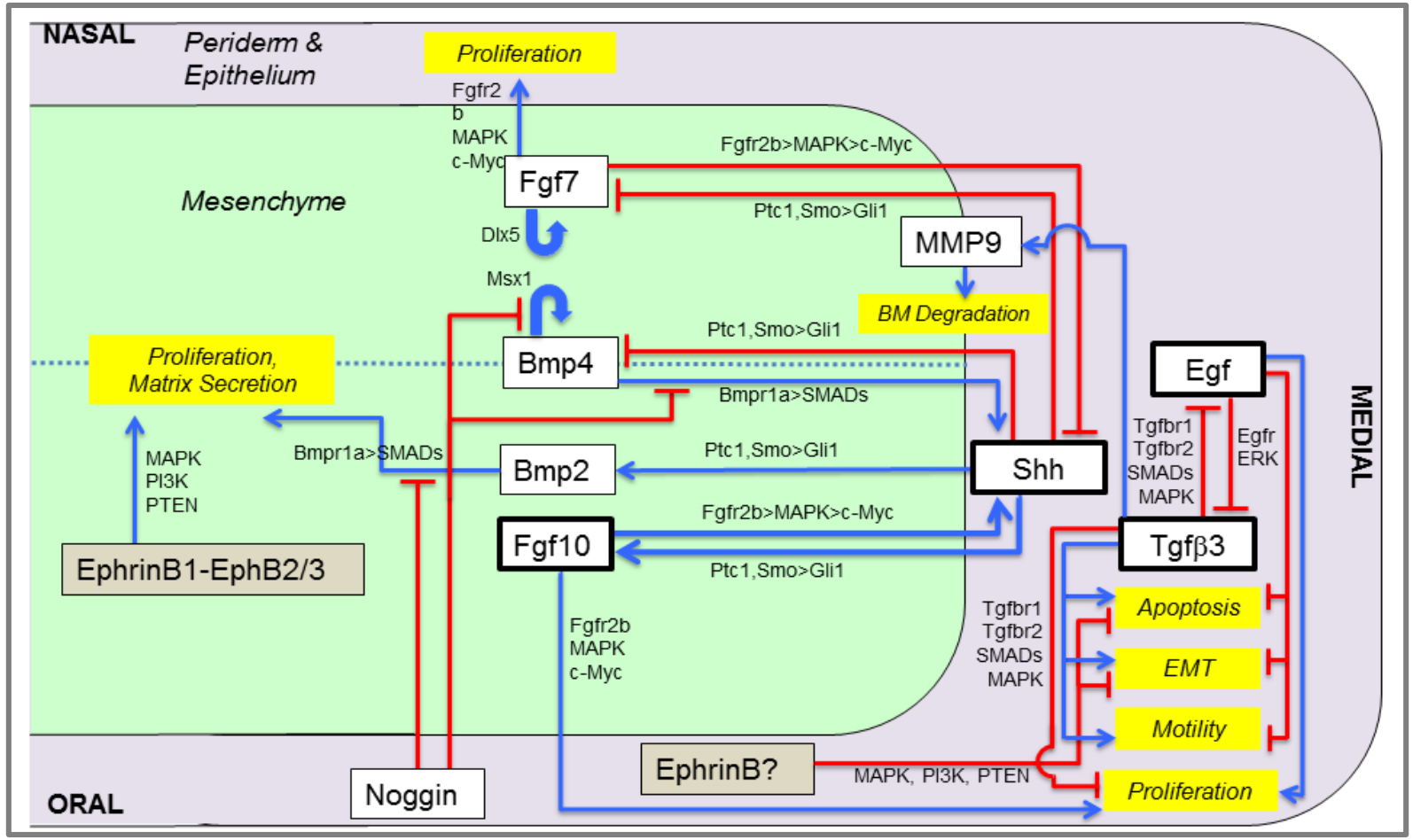


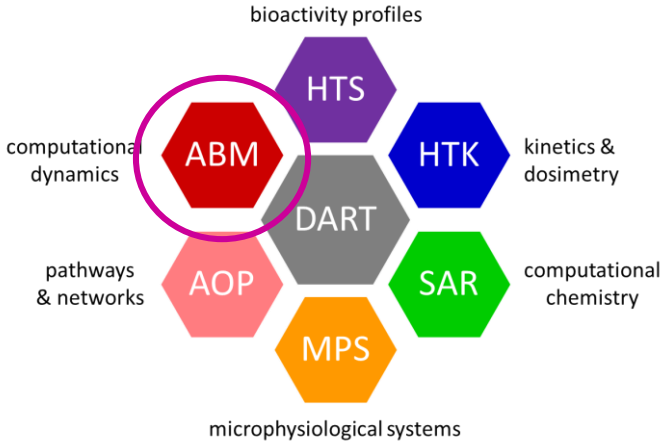
Chemical	Perturbation Days 0-2	Perturbation Days 0-4	Cytotoxicity
TCDD			
All-Trans Retinoic Acid (ATRA)		✓	✓
Dexamethasone			
Corticosterone			
Triamcinolone		✓	
Valproic Acid	✓		
Fluconazole			
Caffeine			
Nicotine			
Tributyltin	✓	✓	✓
Triadimefon			
Theophylline	✓		

- 2,400 spheroids per batch (120 wells per week) to assess tissue fusion in a human cell-based system.
- Process is sensitive to pharma compounds acting on various pathways (EGF, IGF, FGF, HGF, BMP);
- Sensitive to chemicals (ATRA, TBT, VPA, Theophylline, Triamcinolone) via viability or epithelial migration.

SOURCE: Belair et al. (2018) Toxicol Sci

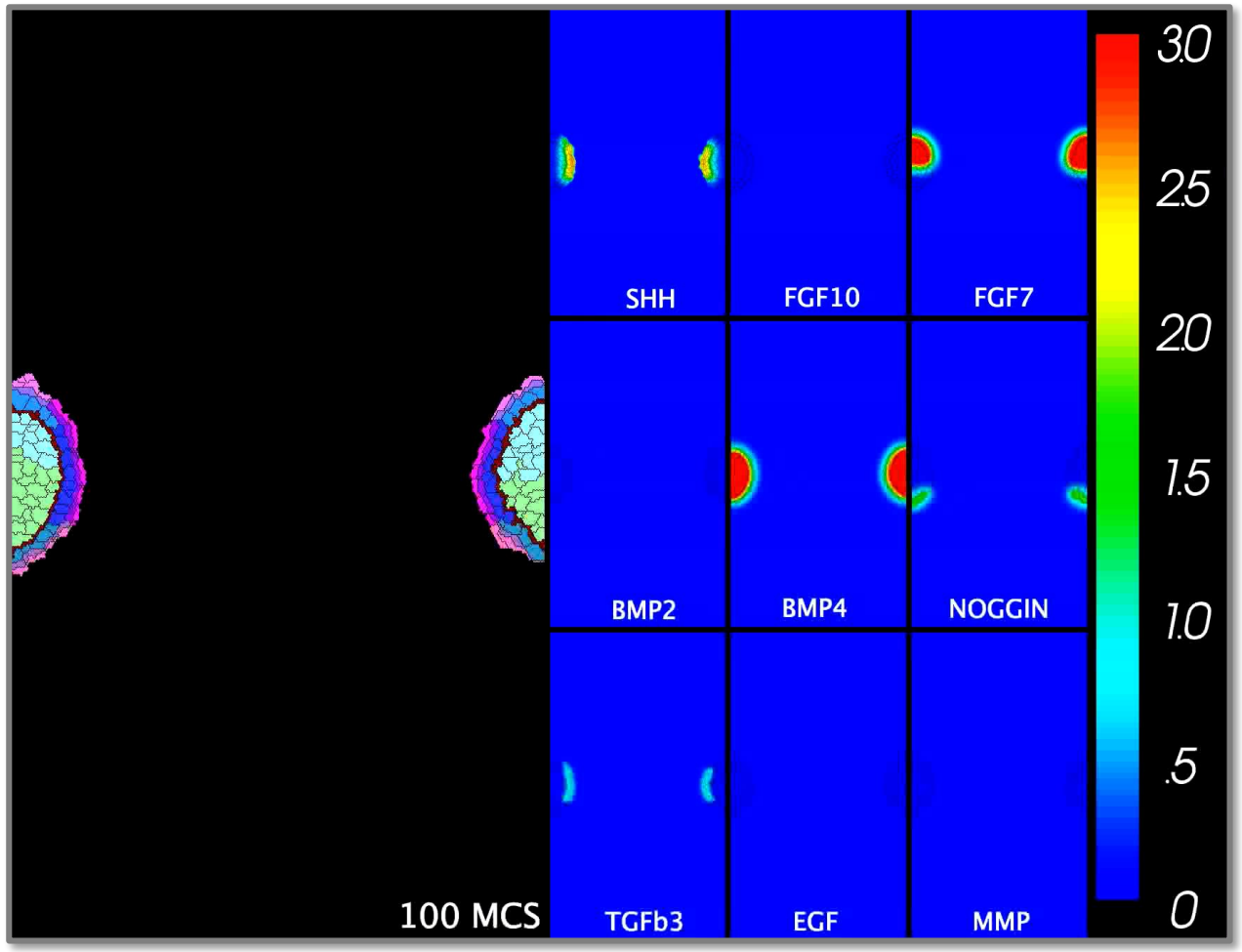
Systems model: palatal outgrowth and fusion

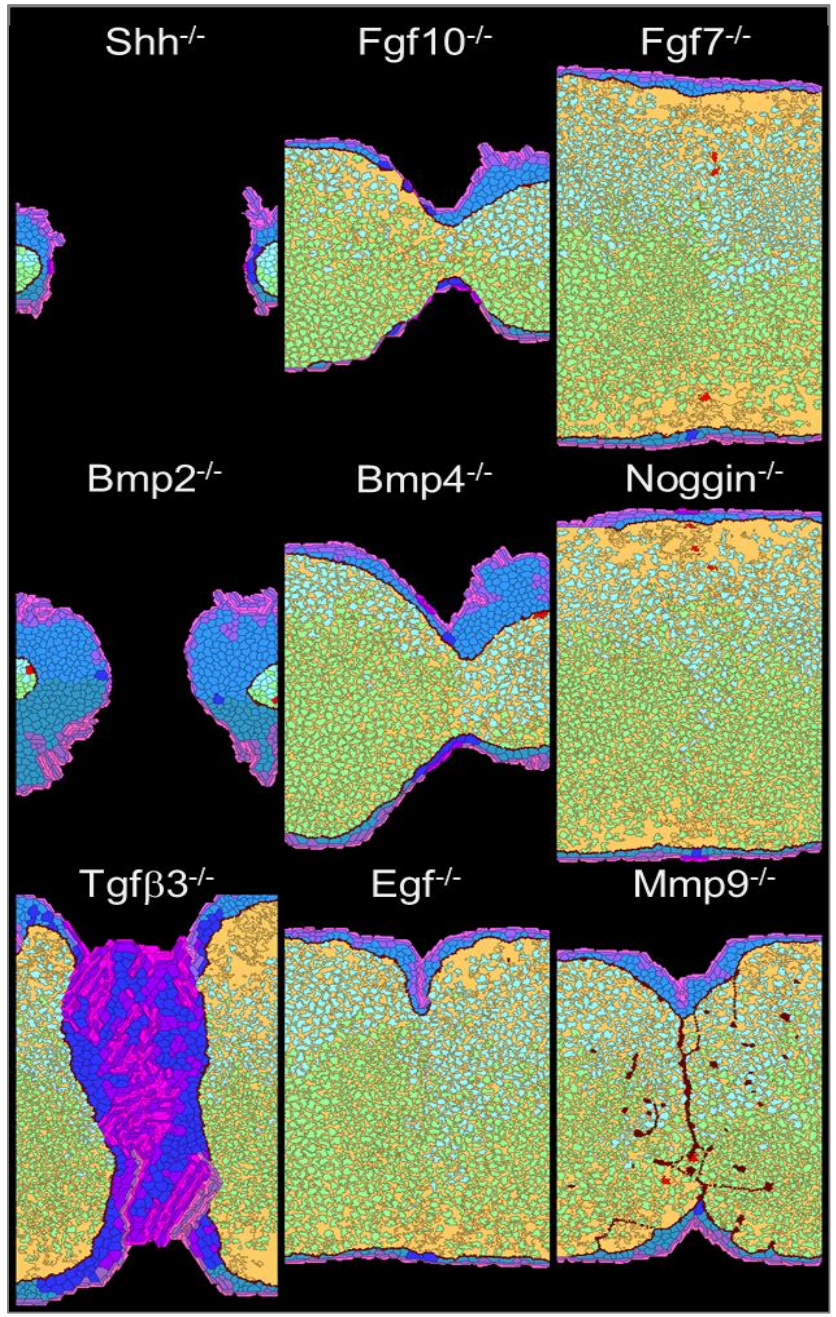




SOURCE: Hutson et al. (2017) Chem Res Toxicol

ABM simulation: hacking the control network

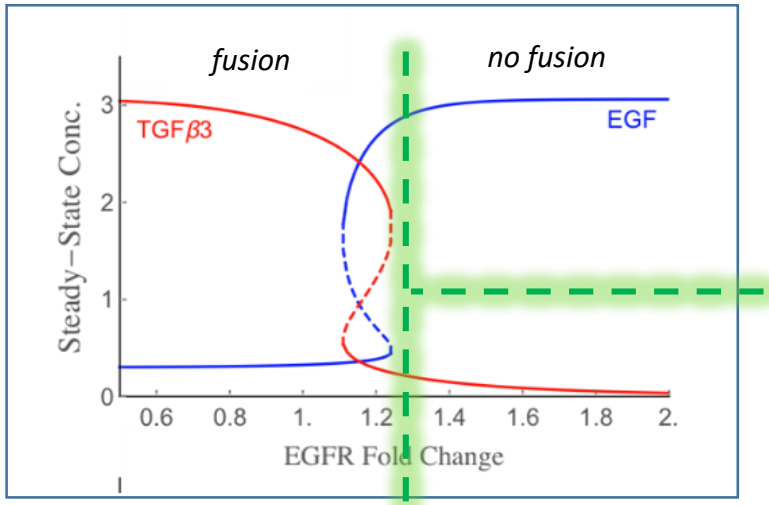




SOURCE: Hutson et al. (2017) Chem Res Toxicol

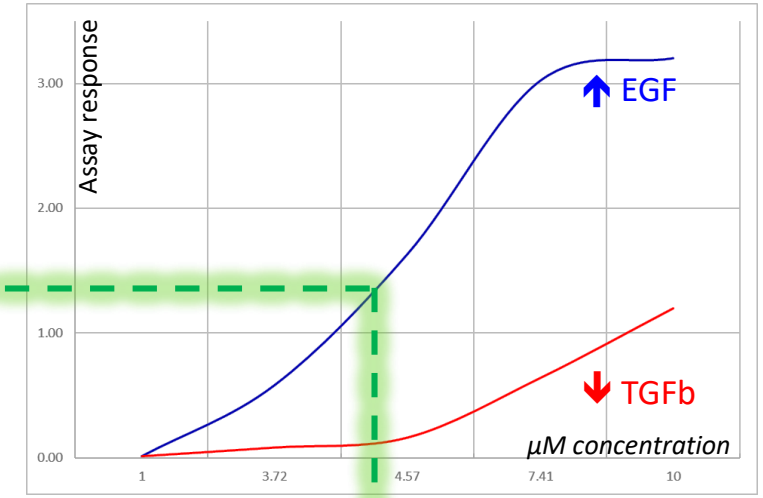
TGF-beta/EGF latch switch: *controls MEE breakdown*

INPUT: switch dynamics



tipping point predicted by computational dynamics (hysteresis switch)

Captan in ToxCast

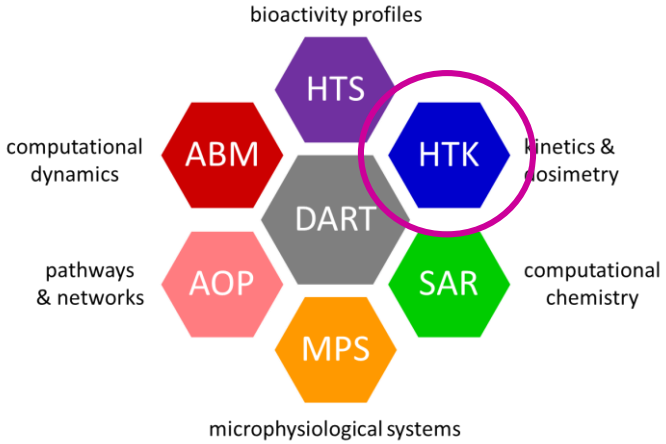


OUTPUT: tipping point mapped to concentration response (4 µM)

Captan in ToxRefDB
NOAEL = 10 mg/kg/day
LOAEL = 30 mg/kg/day

human HTTK model
2.39 mg/kg/day would achieve a steady state of 4 µM in fetal plasma

httk workflow



in vitro or *in silico*
model input parameter estimates (e.g., *fup*, *Clint*)

Real-Life Exposure (mg/kg)

MOE

Risk Prioritization for DevTox

External Dose (mg/kg)

Forward Dosimetry

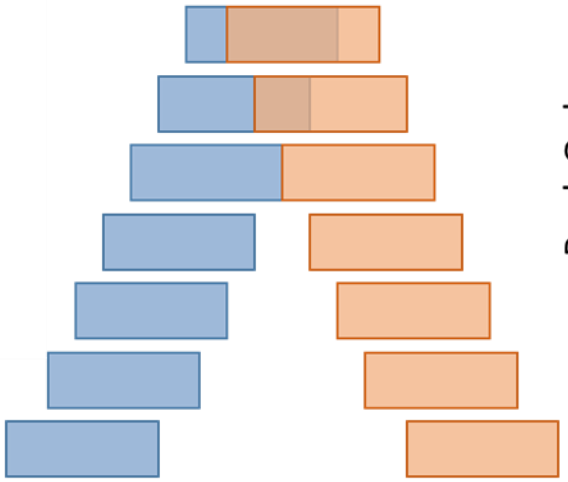
Reverse Dosimetry

Internal Dose (µM)

(e.g. Plasma or Target-Site Concentrations)

in vitro assay

Model Endpoint Estimates



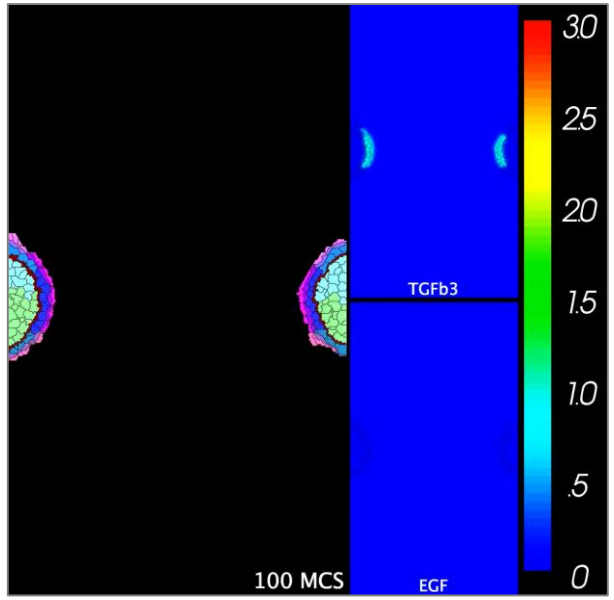
Rank Order

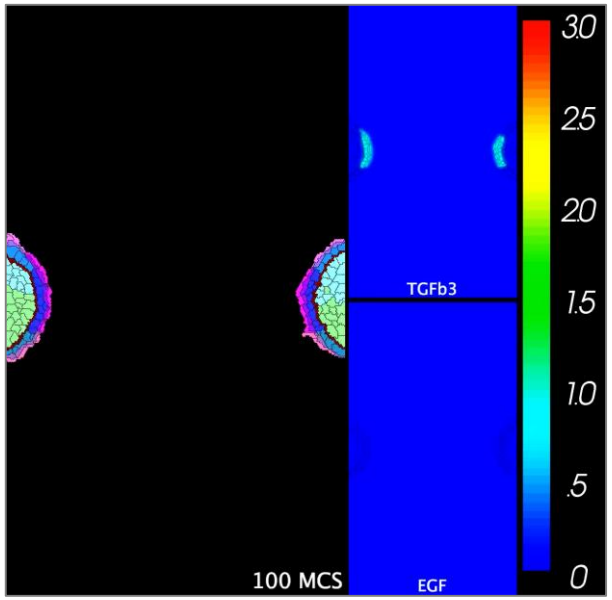
HTTK_PBTK/ Pregnancy Model

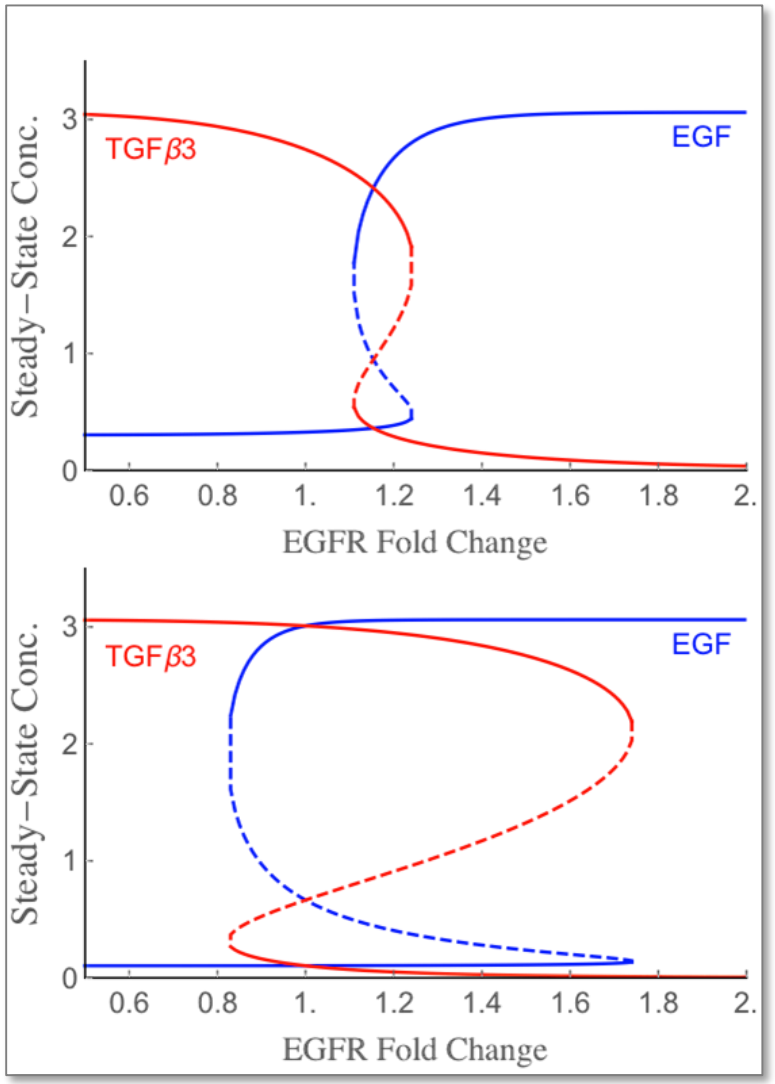
Tipping point can be translated using a 'human pregnancy PBPK model' to infer predicted margins of exposure.

SOURCE: A Lumen (FDA), N Kleinstreuer (NTP)

Messin' with the switch: *two scenarios for bistable dynamics*







Narrow hysteresis:
less resilient but reversible

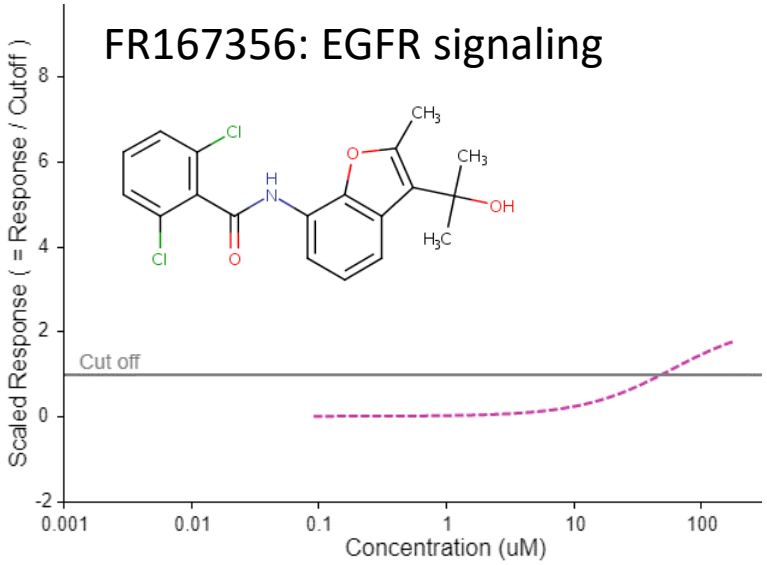
Broad hysteresis:
more resilient but irreversible

Agent-Based Models (ABMs):

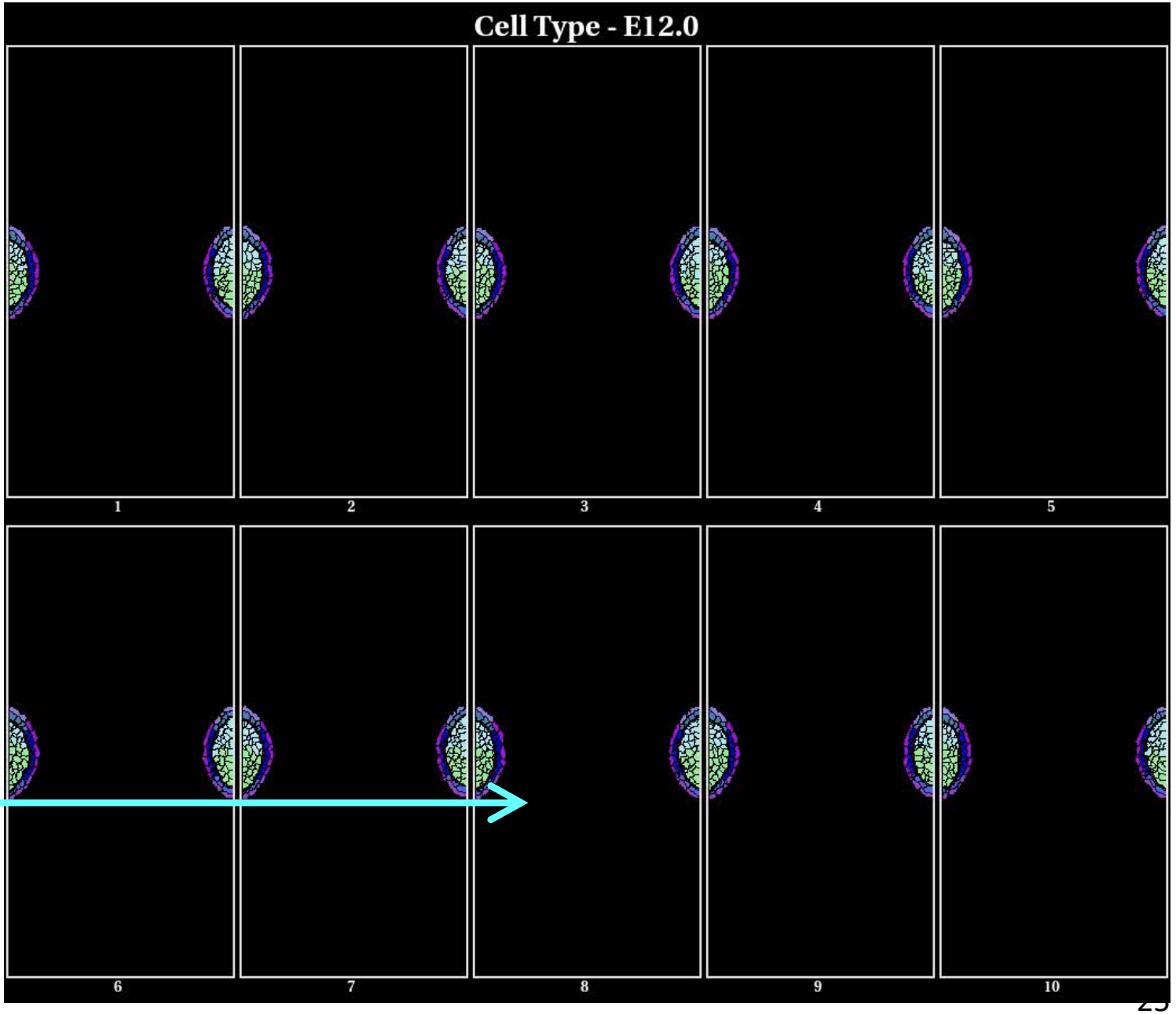
Multicellular simulation is 3R's compliant!

- reconstruct priority systems of embryonic development cell-by-cell and interaction-by-interaction (*emergence*);
- execute tissue simulations that advance through critical determinants of phenotype (*self-organizing phenotypes*);
- simulate *in vitro* data under various *in vivo* scenarios - dose or stage response, critical pathways, non-chemical stressors, etc (*dynamics*);
- probabilistic rendering of where, when and how a defect might occur under different exposure scenarios (*mechanistic interpretation*).

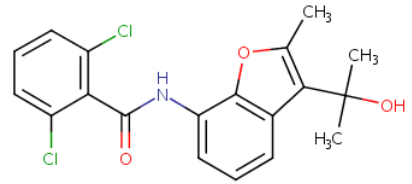
Simulated dose-response

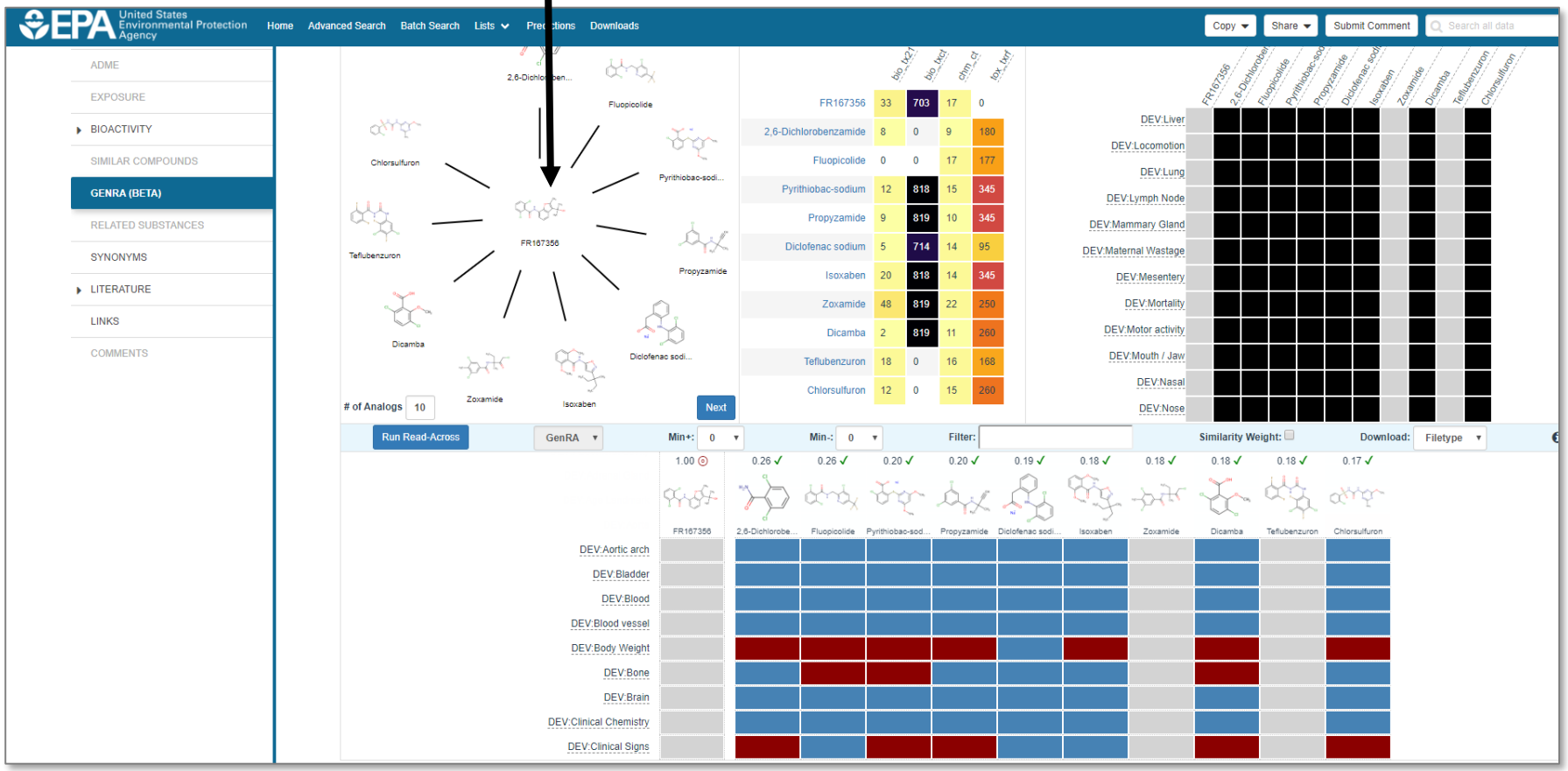


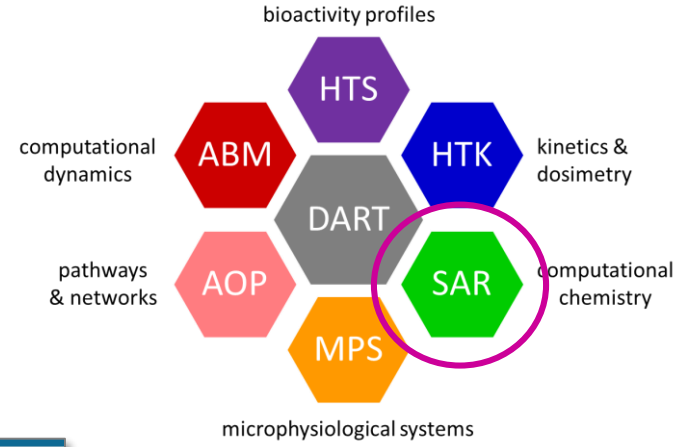
Tipping point predicted in topological context



Read across: structural neighbors to the query





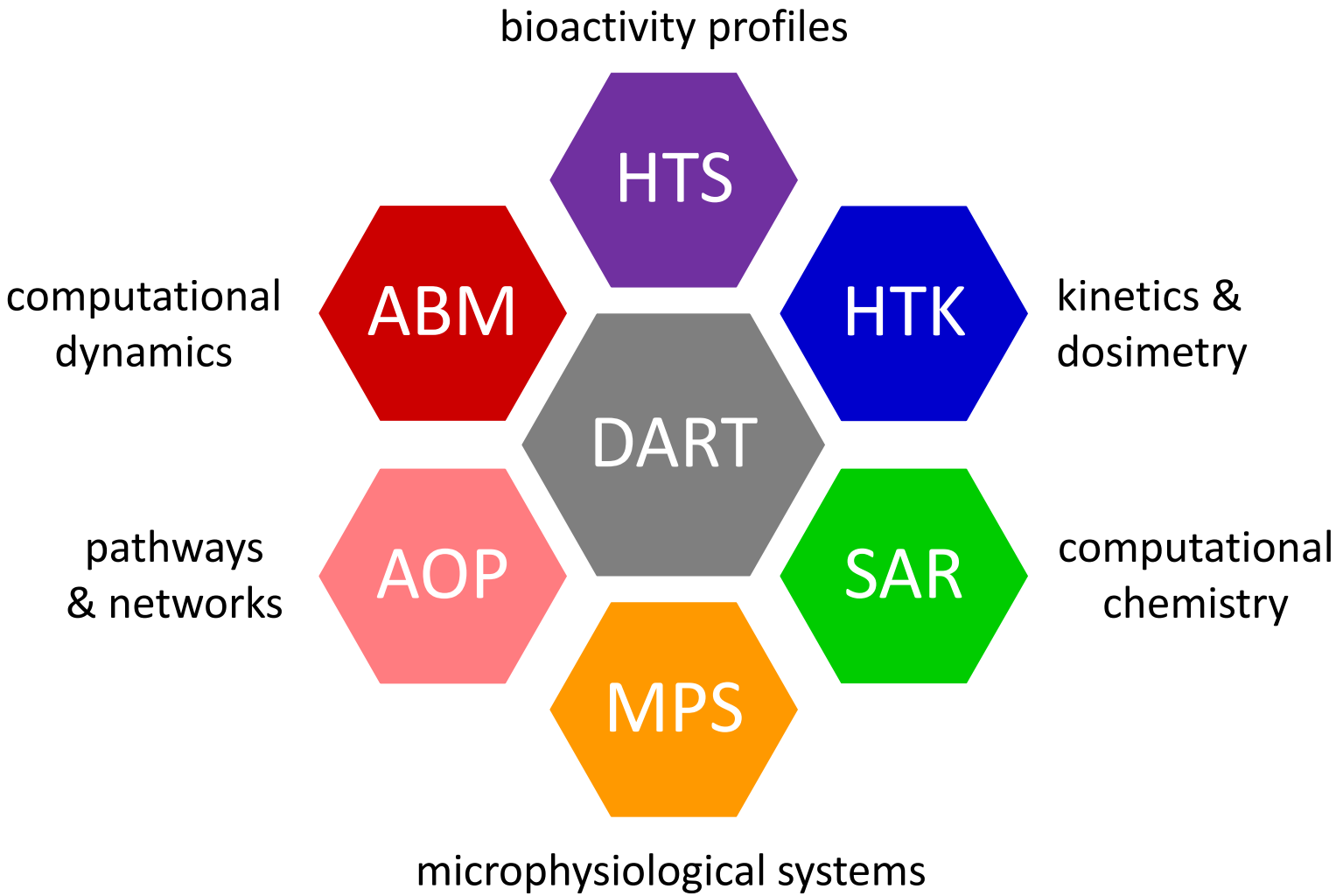


- **FR 167356** is a selective inhibitor of osteoclast vacuolar H+-ATPase (V-ATPase)
- high potency against osteoclast V-ATPase and low potency against lysosomal V-ATPase
- proposed as a drug target in lytic bone diseases due osteoclast V-ATPase selectivity
- No ReproTox, DevTox, Chronic Tox, Neurotox, … available in open database or literature

1	Abstract Sifter	Landscape View						
2			Update Article Counts	View / hide queries	Heat Map by column	Heat Map by row		
3			5684	(reproduction AND (toxicity OR abnormal OR adverse effects))	nital, Hereditary, and Neonatal Diseases and Abnormalities[	palate OR palate/ drug effects) not surgery Not operati	bone AND development	
4	DSSTOX link to Dashboard	Preferred Name	Chemical / entity query	ReproTox	DevTox	CP	bone	
5		Indomethacin	Indomethacin	482	468	7	174	
6	DTXSID3037208	Diclofenac sodium	15307-79-6 OR Diclofenac sodium OR Diclofenac	143	37	1	18	
7	DTXSID4024018	Dicamba	1918-00-9 OR Dicamba	9	1	0	0	
8	DTXSID1048174	FR167356	174185-16-1 OR FR167356 OR FR 167356 OR "FR 167356"[nm]	0	0	0	4	
9	DTXSID7022170	2,6-Dichlorobenzami	2008-58-4 OR 2,6-Dichlorobenzamide	0	0	0	0	
10	DTXSID7034624	Fluopicolide	239110-15-7 OR Fluopicolide	0	0	0	0	
11	DTXSID8032673	Pyrithiobac-sodium	123343-16-8 OR Pyrithiobac-sodium OR pyrithiobac sodium	0	0	0	0	
12	DTXSID2020420	Propyzamide	23950-58-5 OR Propyzamide OR pronamide	1	0	0	0	
13	DTXSID8024159	Isoxaben	82558-50-7 OR Isoxaben	1	0	0	0	
14	DTXSID9032581	Zoxamide	156052-68-5 OR Zoxamide	0	0	0	0	
15	DTXSID6042440	Teflubenzuron	83121-18-0 OR Teflubenzuron	6	0	0	0	
16	DTXSID7023980	Chlorsulfuron	64902-72-3 OR Chlorsulfuron	0	0	0	0	
17	DTXSID4032619	Halofenozide	112226-61-6 OR Halofenozide OR N-4-chlorobenzoyl-N'-benzoyl-N'-tert-l	5	0	0	0	
18	DTXSID0034774	Noviflumuron	121451-02-3 OR Noviflumuron	0	0	0	0	
19	DTXSID7052862	Cumyluron	99485-76-4 OR Cumyluron	0	0	0	0	
20	DTXSID4047672	Flubendiamide	272451-65-7 OR Flubendiamide	5	0	0	0	
21	DTXSID3032628	Methoxyfenozide	161050-58-4 OR Methoxyfenozide	11	0	0	0	
22								

SOURCE: Nancy Baker, NCCT

IATA: computational synthesis and integration



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Nathan Rush - NCCT
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