



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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Virtual Tissue Models (VTM) project

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Problem statement

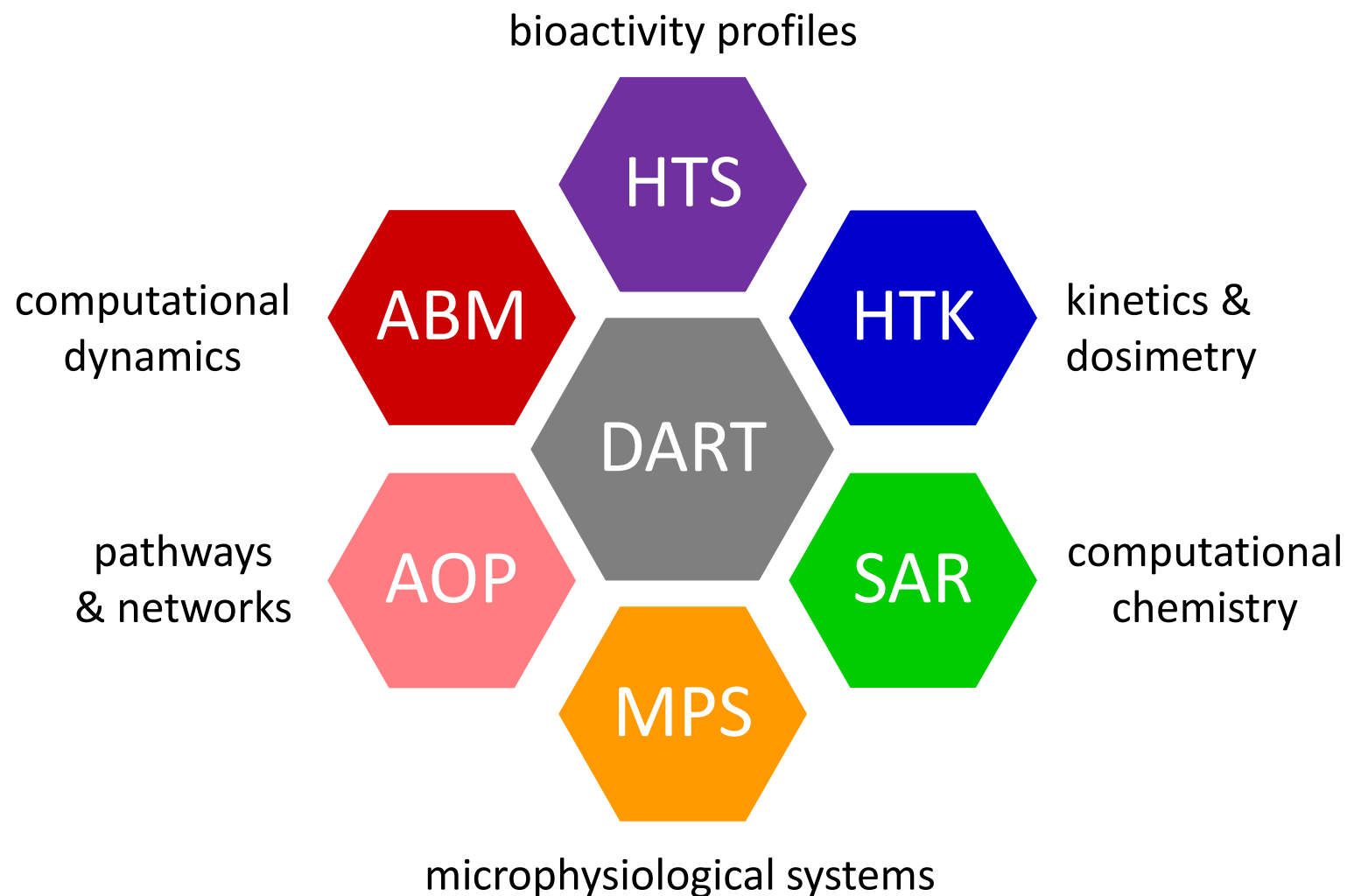
- Chemical exposure to a pregnant woman has the potential to affect her unborn child, leading to adverse birth outcomes and/or risks to early child development.
- Traditional animal-based methods for assessing prenatal developmental toxicity (OECD TG 414) expose pregnant rats and/or rabbits during organogenesis 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 ...

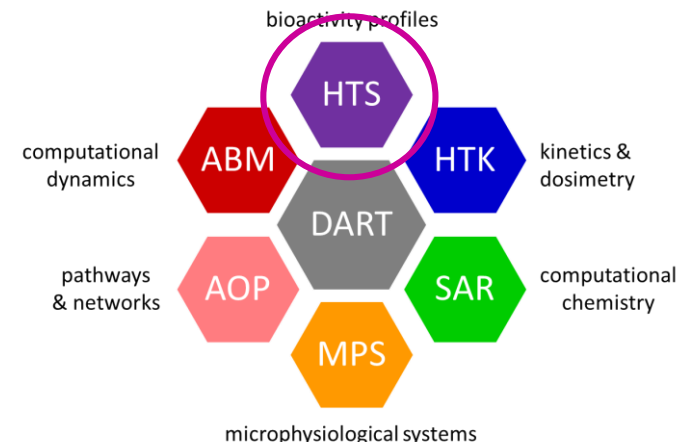
- Many alternative methods for developmental toxicity have been evaluated for 20 years; however, their application as NAMs in a regulatory setting is still poorly defined.
- IATAs can help focus DART resources by strategically targeting developmental hazards predicted by alternative *in vitro* assays 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 positioned to translate AOP-based IATAs predictively and mechanistically with regards to anatomical development.

IATA: computational synthesis and integration



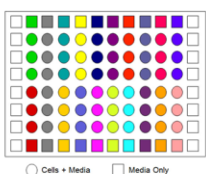
ToxCast STM platform: devTOX^{qP} assay from Stemina

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

* Plate Maps



1323 samples

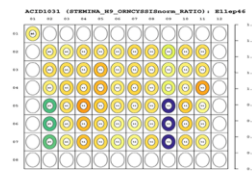
731 records
single conc.

334 records
8-conc. response

1065 structures

985,338
data points

Virtual Plates



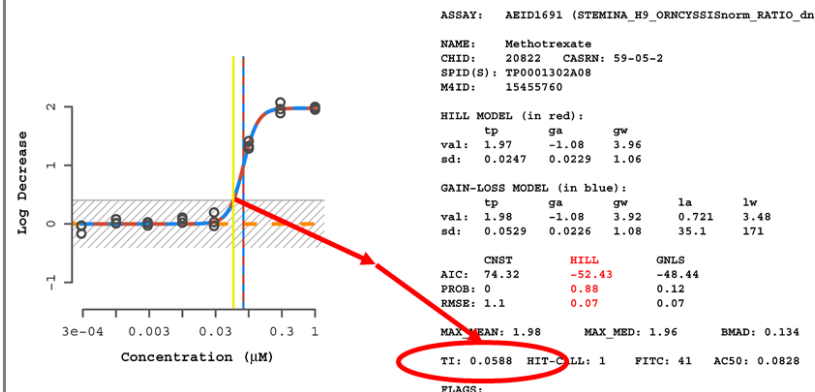
72,198
data points

Level-0
Level-1
Level-2
Level-3
Level-4
Level-5
Level-6

tcpl

invitrodb

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 models¹ 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).

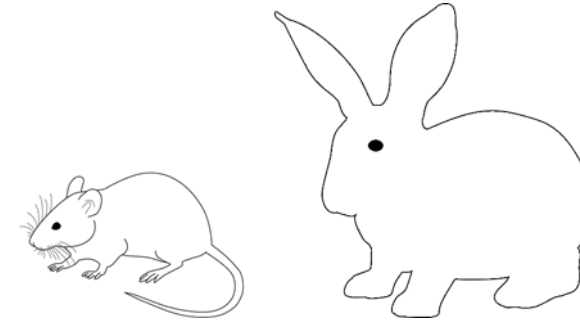
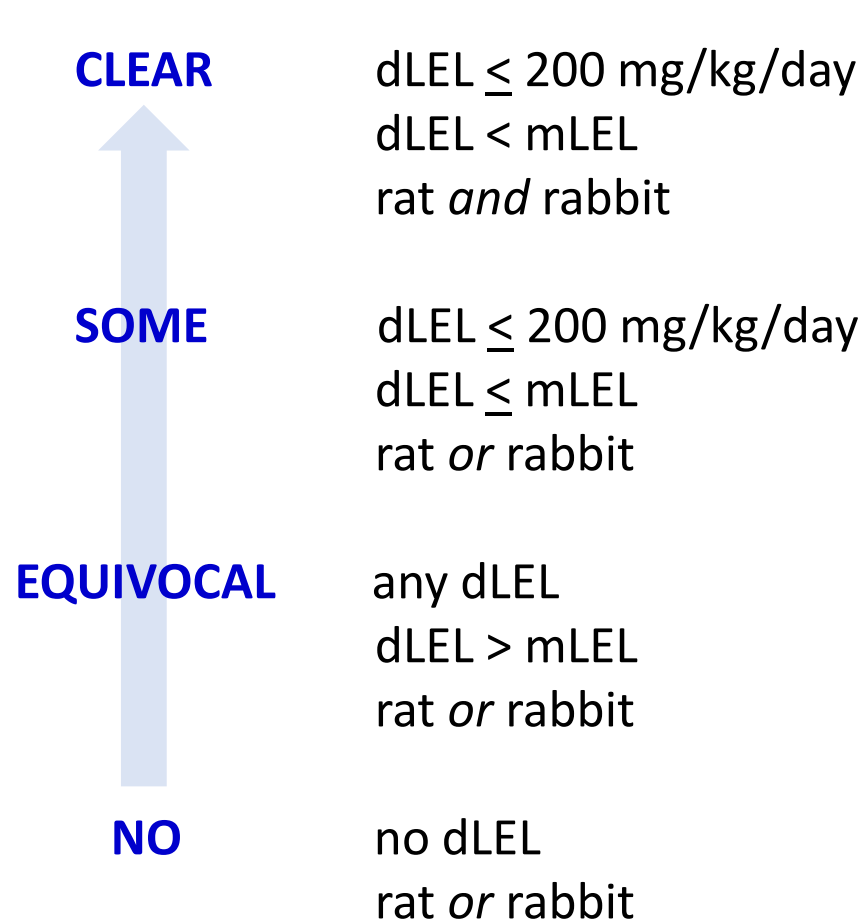
How does the STM prediction do with ToxRefDB (v1) prenatal developmental toxicity studies?

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

SOURCE: NCCT, manuscript in preparation

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	Phenobarbital 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)	

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



- 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 FP FN TN n sensitivity specificity PPV NPV ACC MCC		17	85	60	35	19
		0	14	37	23	9
		9	217	127	51	11
		16	116	208	176	88
		42	432	432	285	127
		0.654	0.281	0.321	0.407	0.633
		1.000	0.892	0.849	0.884	0.907
		1.000	0.859	0.619	0.603	0.679
		0.640	0.348	0.621	0.775	0.889
		78.6%	46.5%	62.0%	74.0%	84.3%
	0.647	0.190	0.202	0.332	0.554	
STM(+)						
		TI ≤ 200 μM	TI ≤ 1000 μM	TI ≤ 200 μM	TI ≤ 200 μM	TI ≤ 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

Applicable Domain: STM results mined against NVS biochemical target profile

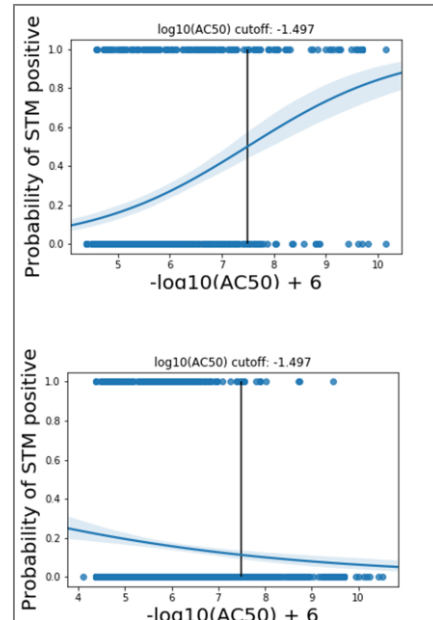
NVS ASIDs selected from
invitroDB v2 (n=422)

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 $\leq$ 31.8
nM correlate
with STM(+)
response

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

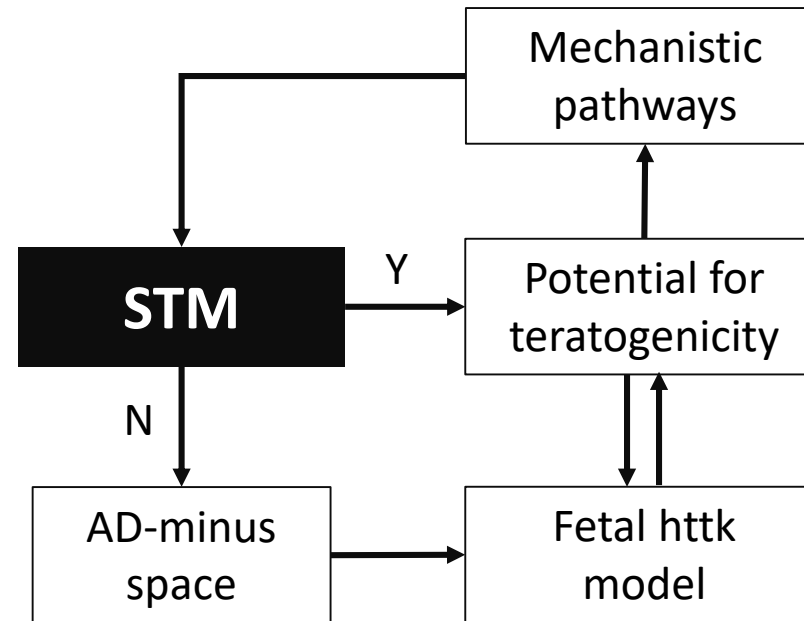
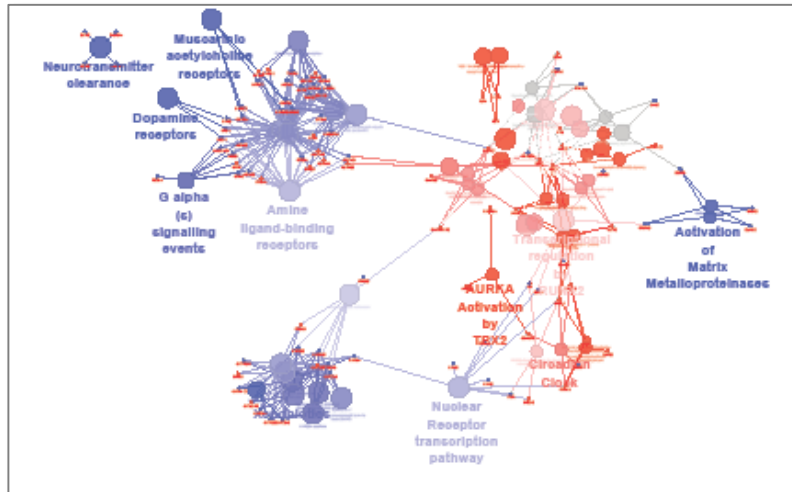
WIS Amary	gen	enrich	z	zRank	z	zRank	z	zRank	z	zRank
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ENR_HNF4A2	ENR_HNF4A2	0.999	2	0.000	44	0.000	2	0.000	2	0.000
ENR_HNF4A3	ENR_HNF4A3	0.999	3	0.000	43	0.000	3	0.000	3	0.000
ENR_HNF4A4	ENR_HNF4A4	0.999	4	0.000	42	0.000	4	0.000	4	0.000
ENR_HNF4A5	ENR_HNF4A5	0.999	5	0.000	41	0.000	5	0.000	5	0.000
ENR_HNF4A6	ENR_HNF4A6	0.999	6	0.000	40	0.000	6	0.000	6	0.000
ENR_HNF4A7	ENR_HNF4A7	0.999	7	0.000	39	0.000	7	0.000	7	0.000
ENR_HNF4A8	ENR_HNF4A8	0.999	8	0.000	38	0.000	8	0.000	8	0.000
ENR_HNF4A9	ENR_HNF4A9	0.999	9	0.000	37	0.000	9	0.000	9	0.000
ENR_HNF4A10	ENR_HNF4A10	0.999	10	0.000	36	0.000	10	0.000	10	0.000
ENR_HNF4A11	ENR_HNF4A11	0.999	11	0.000	35	0.000	11	0.000	11	0.000
ENR_HNF4A12	ENR_HNF4A12	0.999	12	0.000	34	0.000	12	0.000	12	0.000
ENR_HNF4A13	ENR_HNF4A13	0.999	13	0.000	33	0.000	13	0.000	13	0.000
ENR_HNF4A14	ENR_HNF4A14	0.999	14	0.000	32	0.000	14	0.000	14	0.000
ENR_HNF4A15	ENR_HNF4A15	0.999	15	0.000	31	0.000	15	0.000	15	0.000
ENR_HNF4A16	ENR_HNF4A16	0.999	16	0.000	30	0.000	16	0.000	16	0.000
ENR_HNF4A17	ENR_HNF4A17	0.999	17	0.000	29	0.000	17	0.000	17	0.000
ENR_HNF4A18	ENR_HNF4A18	0.999	18	0.000	28	0.000	18	0.000	18	0.000
ENR_HNF4A19	ENR_HNF4A19	0.999	19	0.000	27	0.000	19	0.000	19	0.000
ENR_HNF4A20	ENR_HNF4A20	0.999	20	0.000	26	0.000	20	0.000	20	0.000
ENR_HNF4A21	ENR_HNF4A21	0.999	21	0.000	25	0.000	21	0.000	21	0.000
ENR_HNF4A22	ENR_HNF4A22	0.999	22	0.000	24	0.000	22	0.000	22	0.000
ENR_HNF4A23	ENR_HNF4A23	0.999	23	0.000	23	0.000	23	0.000	23	0.000
ENR_HNF4A24	ENR_HNF4A24	0.999	24	0.000	22	0.000	24	0.000	24	0.000
ENR_HNF4A25	ENR_HNF4A25	0.999	25	0.000	21	0.000	25	0.000	25	0.000
ENR_HNF4A26	ENR_HNF4A26	0.999	26	0.000	20	0.000	26	0.000	26	0.000
ENR_HNF4A27	ENR_HNF4A27	0.999	27	0.000	19	0.000	27	0.000	27	0.000
ENR_HNF4A28	ENR_HNF4A28	0.999	28	0.000	18	0.000	28	0.000	28	0.000
ENR_HNF4A29	ENR_HNF4A29	0.999	29	0.000	17	0.000	29	0.000	29	0.000
ENR_HNF4A30	ENR_HNF4A30	0.999	30	0.000	16	0.000	30	0.000	30	0.000
ENR_HNF4A31	ENR_HNF4A31	0.999	31	0.000	15	0.000	31	0.000	31	0.000
ENR_HNF4A32	ENR_HNF4A32	0.999	32	0.000	14	0.000	32	0.000	32	0.000
ENR_HNF4A33	ENR_HNF4A33	0.999	33	0.000	13	0.000	33	0.000	33	0.000
ENR_HNF4A34	ENR_HNF4A34	0.999	34	0.000	12	0.000	34	0.000	34	0.000
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ENR_HNF4A37	ENR_HNF4A37	0.999	37	0.000	9	0.000	37	0.000	37	0.000
ENR_HNF4A38	ENR_HNF4A38	0.999	38	0.000	8	0.000	38	0.000	38	0.000
ENR_HNF4A39	ENR_HNF4A39	0.999	39	0.000	7	0.000	39	0.000	39	0.000
ENR_HNF4A40	ENR_HNF4A40	0.999	40	0.000	6	0.000	40	0.000	40	0.000
ENR_HNF4A41	ENR_HNF4A41	0.999	41	0.000	5	0.000	41	0.000	41	0.000
ENR_HNF4A42	ENR_HNF4A42	0.999	42	0.000	4	0.000	42	0.000	42	0.000
ENR_HNF4A43	ENR_HNF4A43	0.999	43	0.000	3	0.000	43	0.000	43	0.000
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ENR_HNF4A46	ENR_HNF4A46	0.999	46	0.000	0	0.000	46	0.000	46	0.000
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ENR_HNF4A50	ENR_HNF4A50	0.999	50	0.000	-4	0.000	50	0.000	50	0.000
ENR_HNF4A51	ENR_HNF4A51	0.999	51	0.000	-5	0.000	51	0.000	51	0.000
ENR_HNF4A52	ENR_HNF4A52	0.999	52	0.000	-6	0.000	52	0.000	52	0.000
ENR_HNF4A53	ENR_HNF4A53	0.999	53	0.000	-7	0.000	53	0.000	53	0.000
ENR_HNF4A54	ENR_HNF4A54	0.999	54	0.000	-8	0.000	54	0.000	54	0.000
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ENR_HNF4A56	ENR_HNF4A56	0.999	56	0.000	-10	0.000	56	0.000	56	0.000
ENR_HNF4A57	ENR_HNF4A57	0.999	57	0.000	-11	0.000	57	0.000	57	0.000
ENR_HNF4A58	ENR_HNF4A58	0.999	58	0.000	-12	0.000	58	0.000	58	0.000
ENR_HNF4A59	ENR_HNF4A59	0.999	59	0.000	-13	0.000	59	0.000	59	0.000
ENR_HNF4A60	ENR_HNF4A60	0.999	60	0.000	-14	0.000	60	0.000	60	0.000
ENR_HNF4A61	ENR_HNF4A61	0.999	61	0.000	-15	0.000	61	0.000	61	0.000
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ENR_HNF4A64	ENR_HNF4A64	0.999	64	0.000	-18	0.000	64	0.000	64	0.000
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ENR_HNF4A68	ENR_HNF4A68	0.999	68	0.000	-22	0.000	68	0.000	68	0.000
ENR_HNF4A69	ENR_HNF4A69	0.999	69	0.000	-23	0.000	69	0.000	69	0.000
ENR_HNF4A70	ENR_HNF4A70	0.999	70	0.000	-24	0.000	70	0.000	70	0.000
ENR_HNF4A71	ENR_HNF4A71	0.999	71	0.000	-25	0.000	71	0.000	71	0.000
ENR_HNF4A72	ENR_HNF4A72	0.999	72	0.000	-26	0.000	72	0.000	72	0.000
ENR_HNF4A73	ENR_HNF4A73	0.999	73	0.000	-27	0.000	73	0.000	73	0.000
ENR_HNF4A74	ENR_HNF4A74	0.999	74	0.000	-28	0.000	74	0.000	74	0.000
ENR_HNF4A75	ENR_HNF4A75	0.999	75	0.000	-29	0.000	75	0.000	75	0.000
ENR_HNF4A76	ENR_HNF4A76	0.999	76	0.000	-30	0.000	76	0.000	76	0.000
ENR_HNF4A77	ENR_HNF4A77	0.999	77	0.000	-31	0.000	77	0.000	77	0.000
ENR_HNF4A78	ENR_HNF4A78	0.999	78	0.000	-32	0.000	78	0.000	78	0.000
ENR_HNF4A79	ENR_HNF4A79	0.999	79	0.000	-33	0.000	79	0.000	79	0.000
ENR_HNF4A80	ENR_HNF4A80	0.999	80	0.000	-34	0.000	80	0.000	80	0.000
ENR_HNF4A81	ENR_HNF4A81	0.999	81	0.000	-35	0.000	81	0.000	81	0.000
ENR_HNF4A82	ENR_HNF4A82	0.999	82	0.000	-36	0.000	82	0.000	82	0.000
ENR_HNF4A83	ENR_HNF4A83	0.999	83	0.000	-37	0.000	83	0.000	83	0.000
ENR_HNF4A84	ENR_HNF4A84	0.999	84	0.000	-38	0.000	84	0.000	84	0.000
ENR_HNF4A85	ENR_HNF4A85	0.999	85	0.000	-39	0.000	85	0.000	85	0.000
ENR_HNF4A86	ENR_HNF4A86	0.999	86	0.000	-40	0.000	86	0.000	86	0.000
ENR_HNF4A87	ENR_HNF4A87	0.999	87	0.000	-41	0.000	87	0.000	87	0.000
ENR_HNF4A88	ENR_HNF4A88	0.999	88	0.000	-42	0.000	88	0.000	88	0.000
ENR_HNF4A89	ENR_HNF4A89	0.999	89	0.000	-43	0.000	89	0.000	89	0.000
ENR_HNF4A90	ENR_HNF4A90	0.999	90	0.000	-44	0.000	90	0.000	90	0.000
ENR_HNF4A91	ENR_HNF4A91	0.999	91	0.000	-45	0.000	91	0.000	91	0.000
ENR_HNF4A92	ENR_HNF4A92	0.999	92	0.000	-46	0.000	92	0.000	92	0.000
ENR_HNF4A93	ENR_HNF4A93	0.999	93	0.000	-47	0.000	93	0.000	93	0.000
ENR_HNF4A94	ENR_HNF4A94	0.999	94	0.000	-48	0.000	94	0.000	94	0.000
ENR_HNF4A95	ENR_HNF4A95	0.999	95	0.000	-49	0.000	95	0.000	95	0.000
ENR_HNF4A96	ENR_HNF4A96	0.999	96	0.000	-50	0.000	96	0.000	96	0.000
ENR_HNF4A97	ENR_HNF4A97	0.999	97	0.000	-51	0.000	97	0.000	97	0.000
ENR_HNF4A98	ENR_HNF4A98	0.999	98	0.000	-52	0.000	98	0.000	98	0.000
ENR_HNF4A99	ENR_HNF4A99	0.999	99	0.000	-53	0.000	99	0.000	99	0.000
ENR_HNF4A100	ENR_HNF4A100	0.999	100	0.000	-54	0.000	100	0.000	100	0.000
ENR_HNF4A101	ENR_HNF4A101	0.999	101	0.000	-55	0.000	101	0.000	101	0.000
ENR_HNF4A102	ENR_HNF4A102	0.999	102	0.000	-56	0.000	102	0.000	102	0.000
ENR_HNF4A103	ENR_HNF4A103	0.999	103	0.000	-57	0.000	103	0.000	103	0.000
ENR_HNF4A104	ENR_HNF4A104	0.999	104	0.000	-58	0.000	104	0.000	104	0.000
ENR_HNF4A105	ENR_HNF4A105	0.999	105	0.000	-59	0.000	105	0.000	105	0.000
ENR_HNF4A106	ENR_HNF4A106	0.999	106	0.000	-60	0.000	106	0.000	106	0.000
ENR_HNF4A107	ENR_HNF4A107	0.999	107	0.000	-61	0.000	107	0.000	107	0.000
ENR_HNF4A108	ENR_HNF4A108	0.999	108	0.000	-62	0.000	108	0.000	108	0.000
ENR_HNF4A109	ENR_HNF4A109	0.999	109	0.000	-63	0.000	109	0.000	109	0.000
ENR_HNF4A110	ENR_HNF4A110	0.999	110	0.000	-64	0.000	110	0.000	110	0.000
ENR_HNF4A111	ENR_HNF4A111	0.999	111	0.000	-65	0.000	111	0.000	111	0.000
ENR_HNF4A112	ENR_HNF4A112	0.999	112	0.000	-66	0.000	112	0.000	112	0.000
ENR_HNF4A113	ENR_HNF4A113	0.999	113	0.000	-67	0.000	113	0.000	113	0.000
ENR_HNF4A114	ENR_HNF4A114	0.999	114	0.000	-68	0.000	114	0.000	1	

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

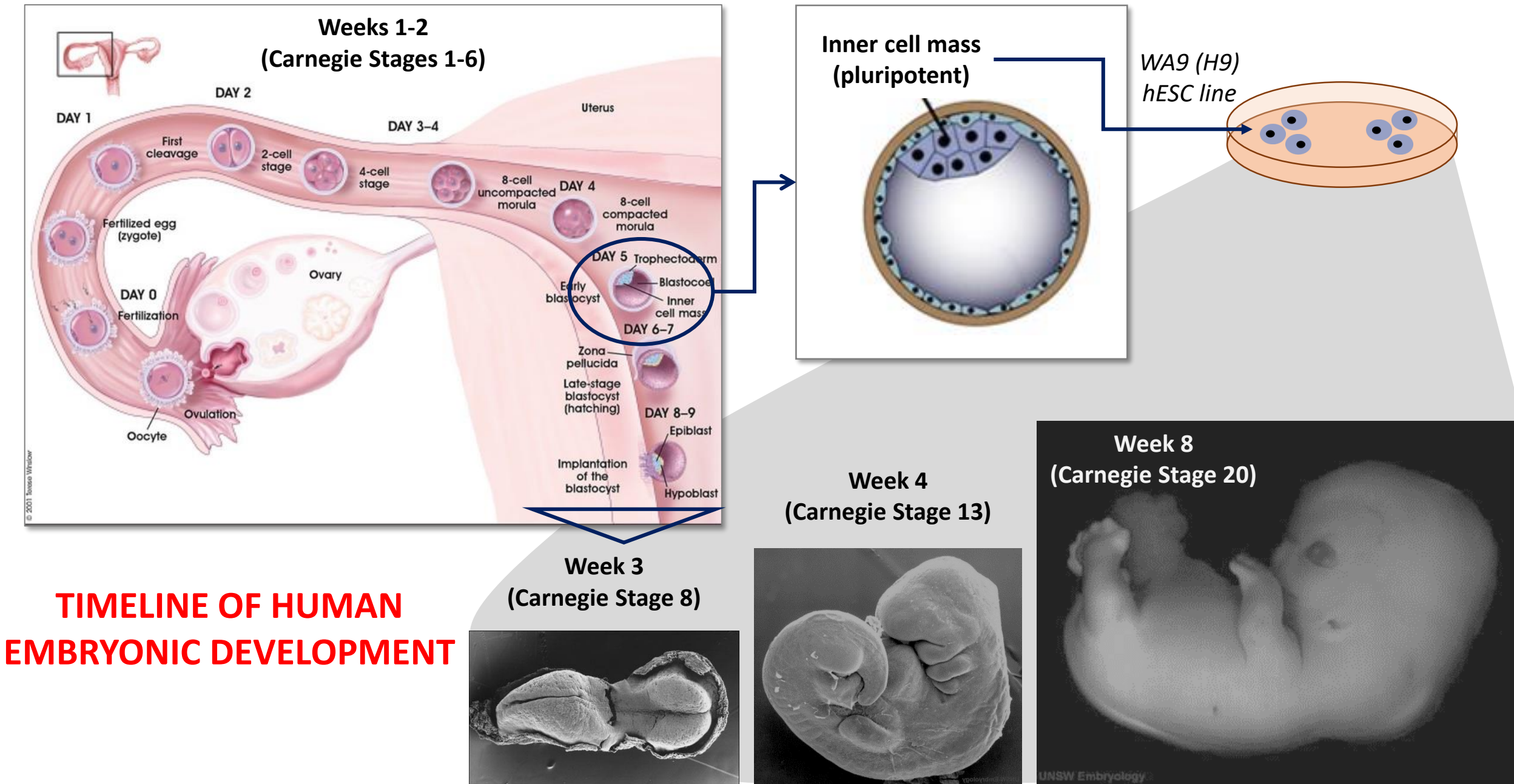


GPCRs (CHRM1/2/3/4/5, HTR2A/2C, DRD4, EDNRA/B, ...)

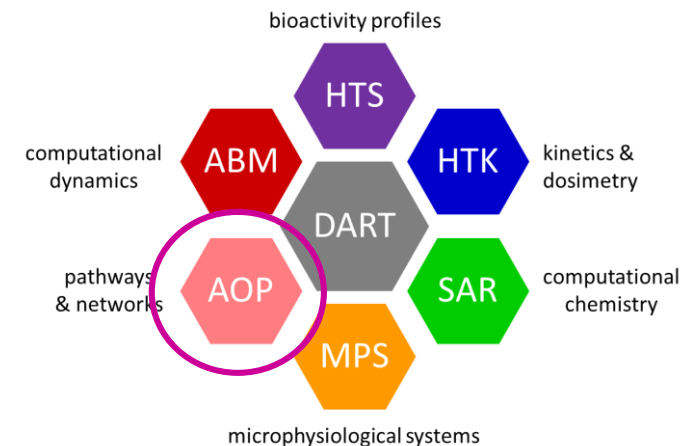
Nuclear receptors (ESR1, PGR, AR, PPARG)

Phosphatases (PTEN, PTPN4/9/11)

-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



AOP-based modeling: cleft palate as an example



ToxCast Chemicals

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

Animal studies

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

AOP clusters

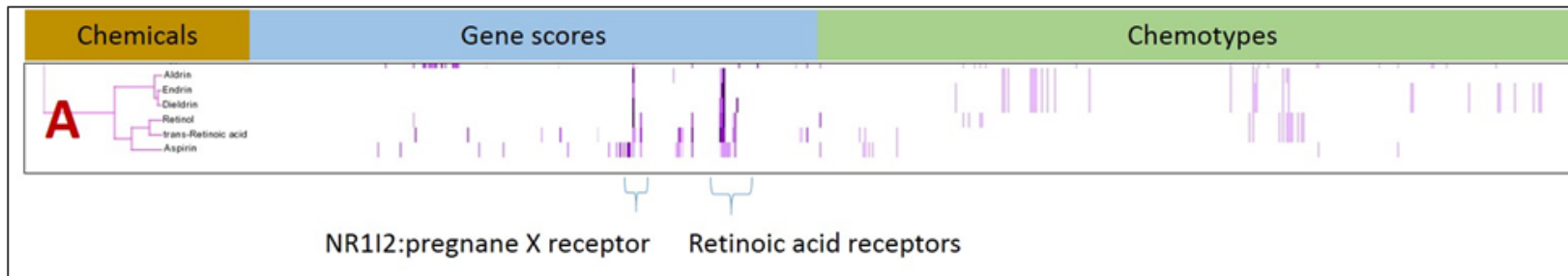
6 mechanistic profiles inferred from integration of HTS data with chemical structure.

Chemicals

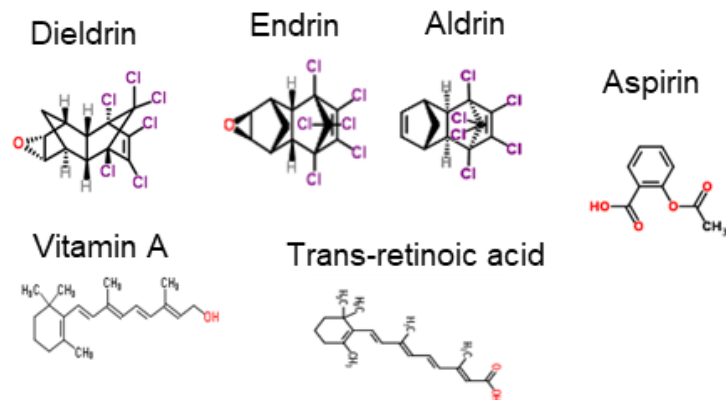
Gene scores

Chemotypes

i)



ii)



iii)

Chemical	Cleft palate? 0/1	Gene Score RARG
Retinol	1	13.5
Dieldrin	1	12.0
Endrin	1	11.9
Aldrin	1	11.6
Bromconazole	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		

A

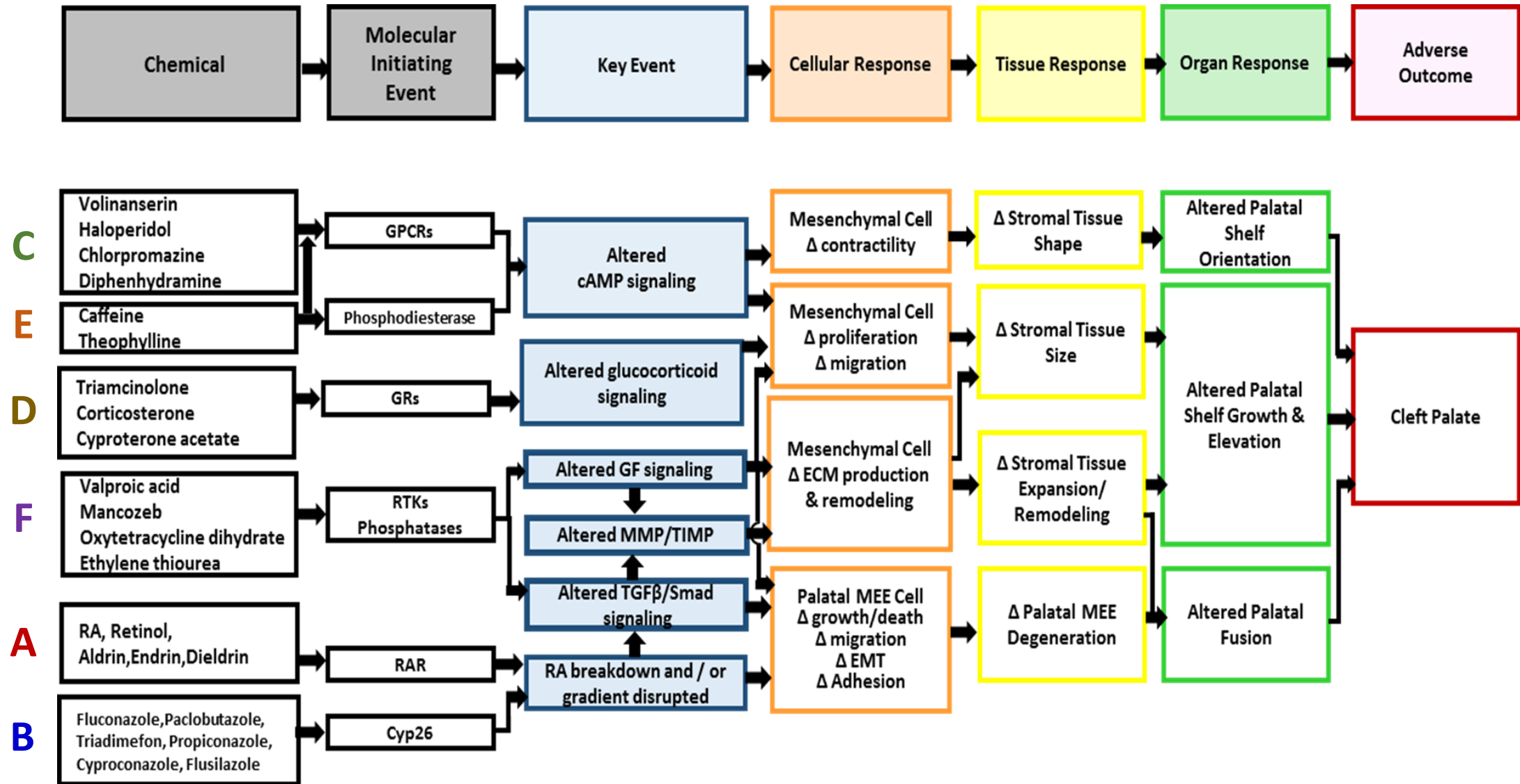
Cyproconazole
Aldrin
Endrin
Dieldrin
Retinol
trans-Retinoic acid
Aspirin

0.00

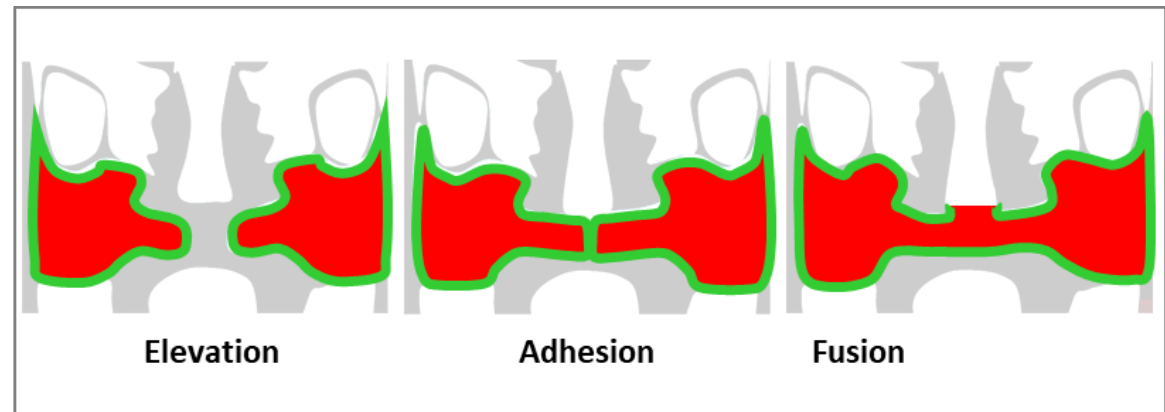
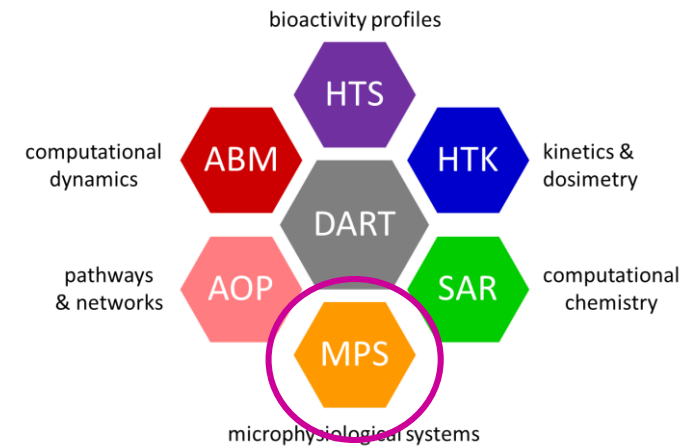
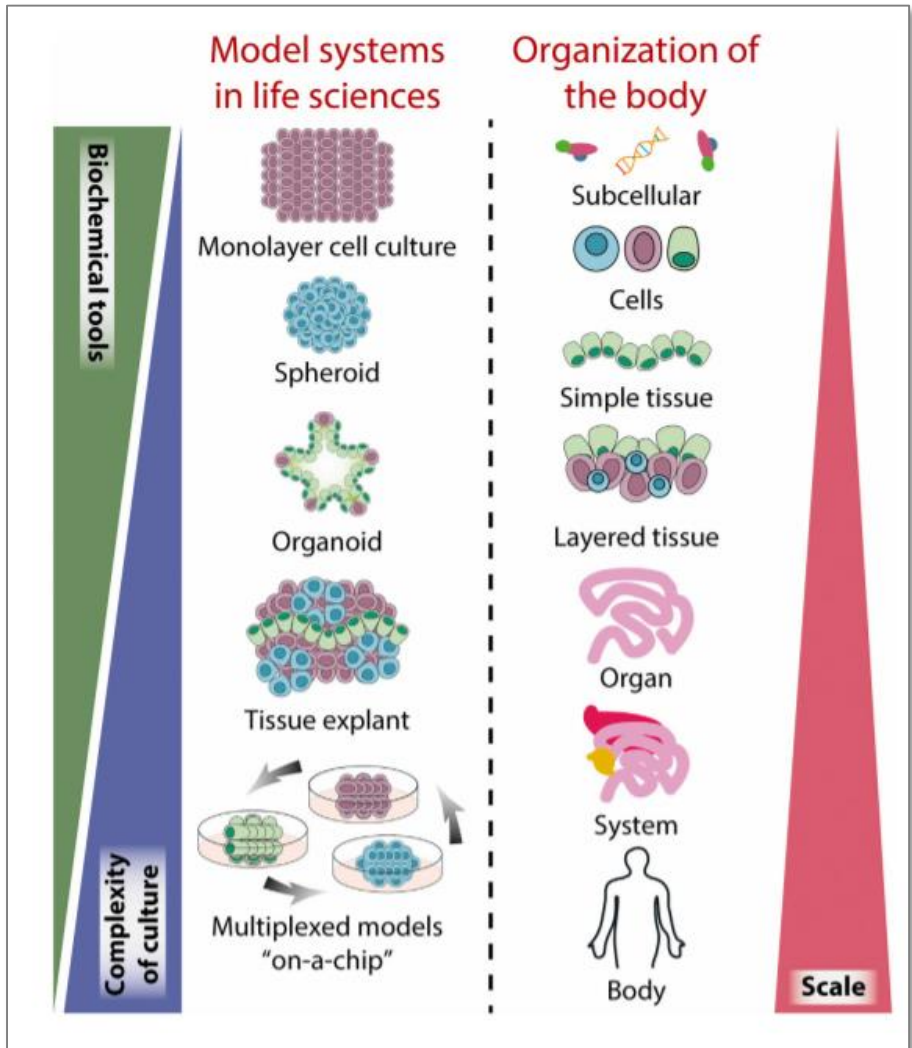
7.40

14.80

AOP clusters: inferred from chemical structure-bioactivity profiles



Microtissue models: reconstructing the system



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

SOURCE: Yin et al. Cell Stem Cell 2016.

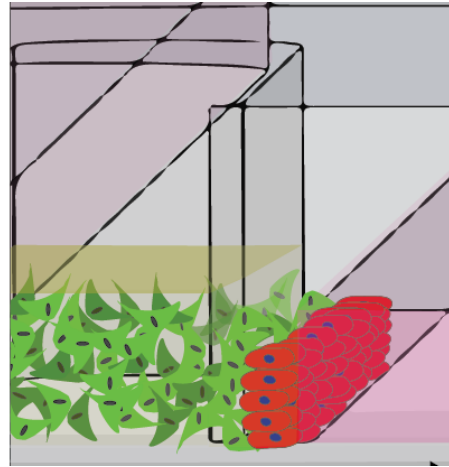
Microphysiological system: reverse-engineering E/M interactions during outgrowth

Microfluidic plates
(40 devices)

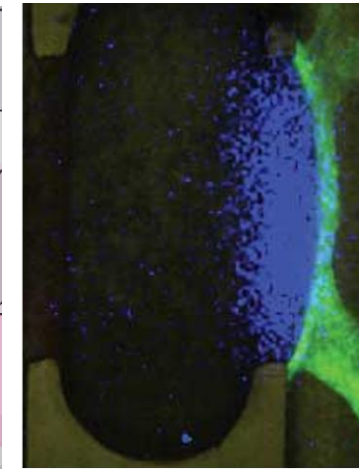


Media
Ports

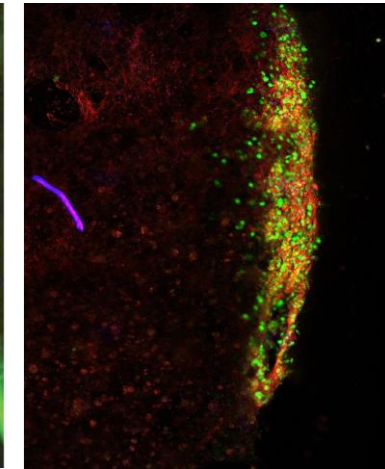
Microtissue
Wells



Gli-luciferase

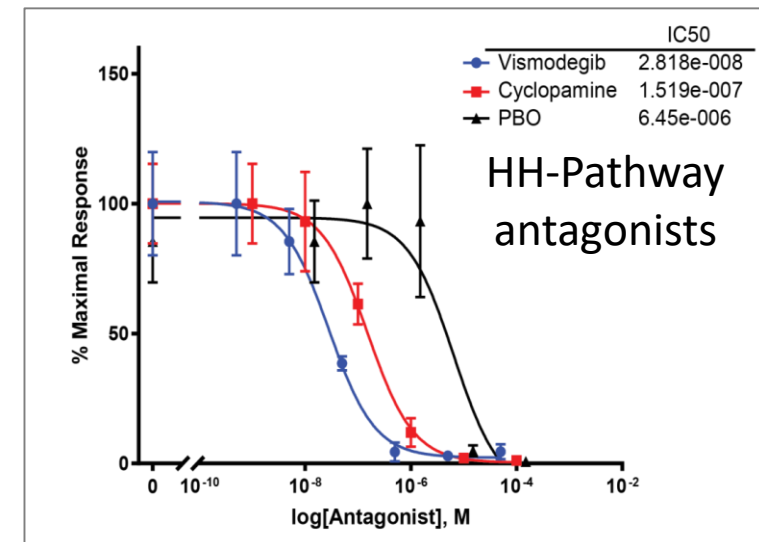


Ki67 proliferation

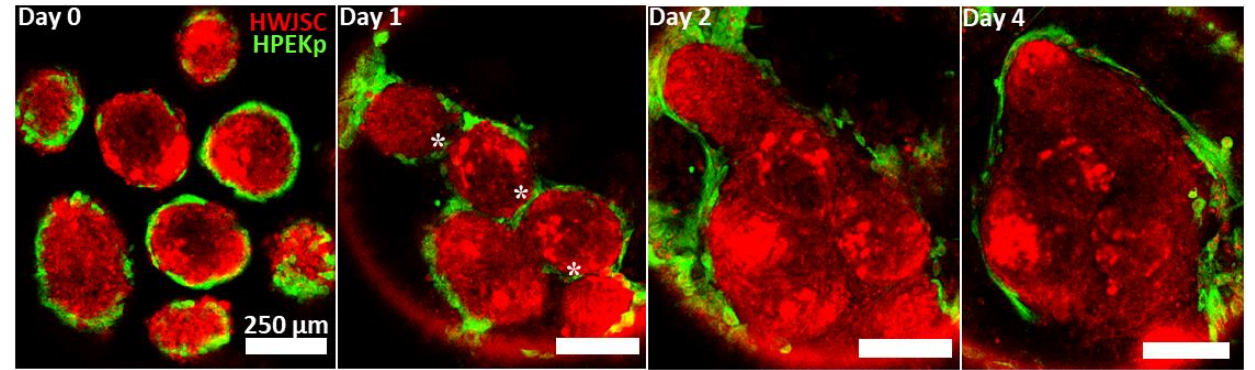
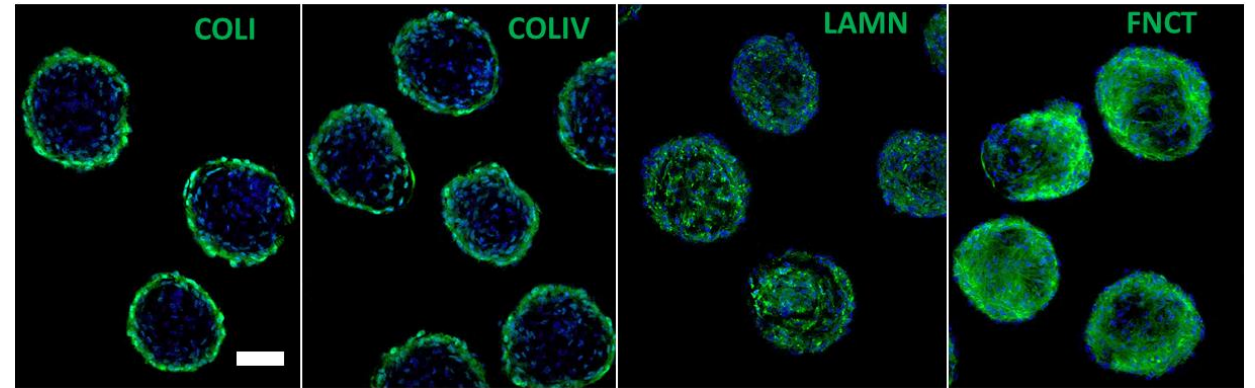
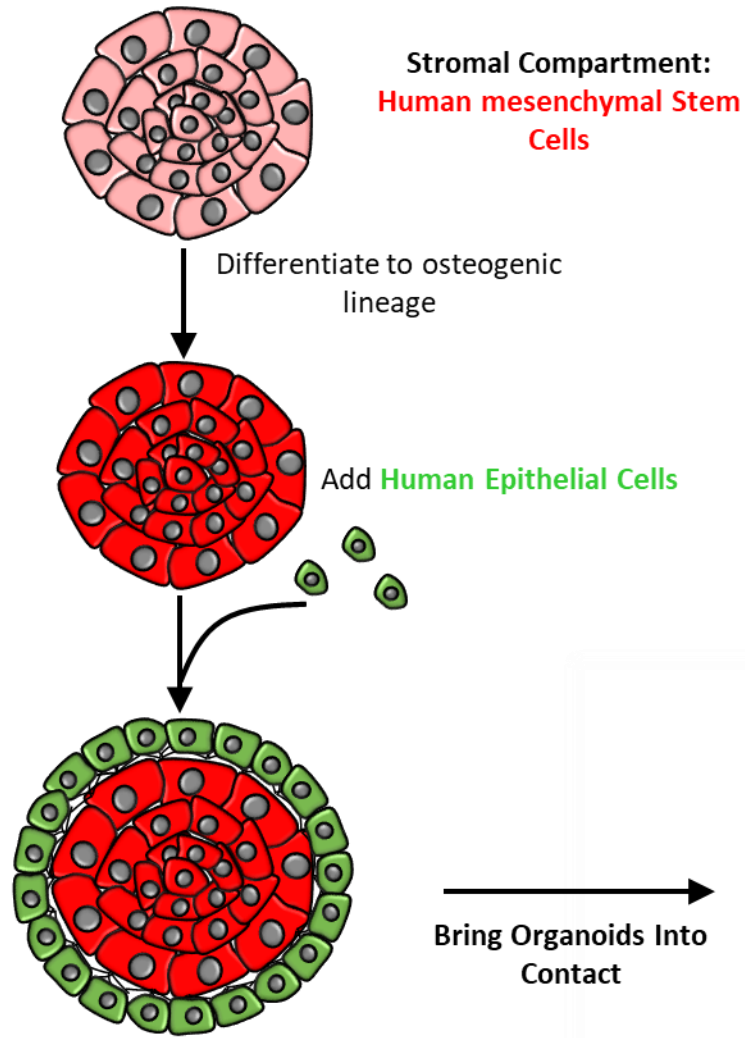


- 3D epithelial / mesenchymal organization
- directed SHH gradient and Gli1-outgrowth
- HTS and HCI amenable
- fluorescent and luminescent readouts

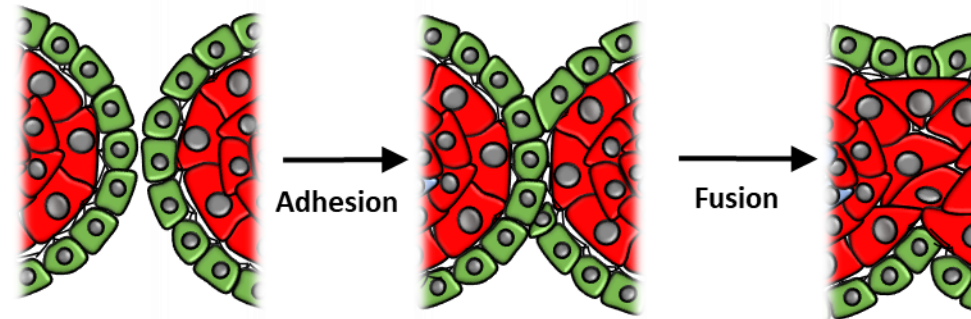
SOURCE: Brian Johnson, U Wisconsin (HMAPS)



Fusion-competent organoids



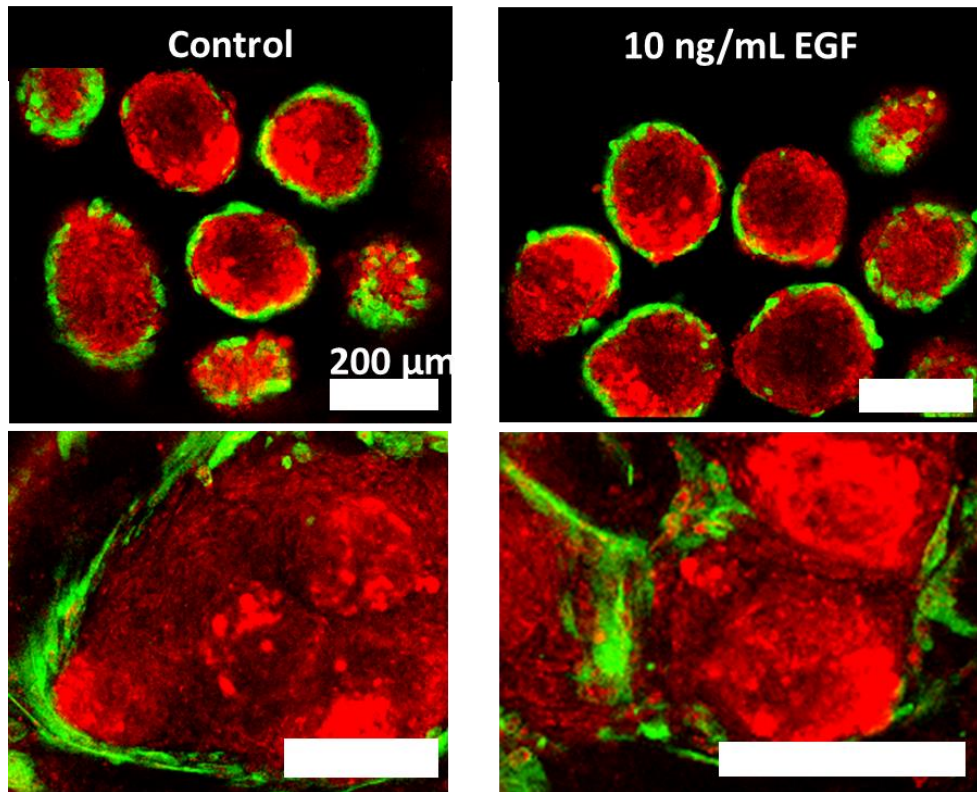
In Vitro Fusion



Monitor Adhesion and Fusion of
Mesenchymal/Epithelial Organoids

SOURCE: Belair et al. (2018) Toxicol Sci

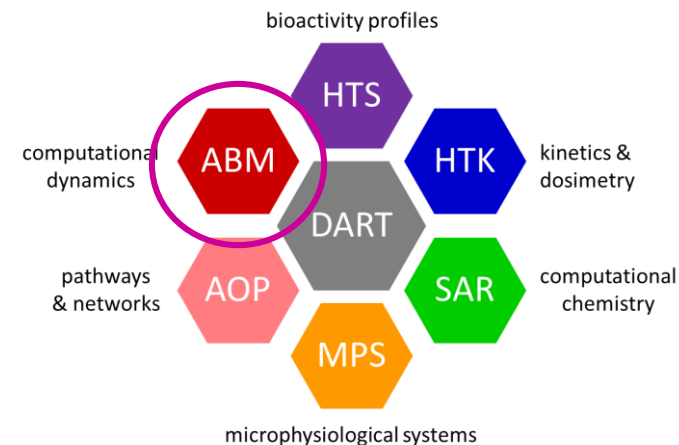
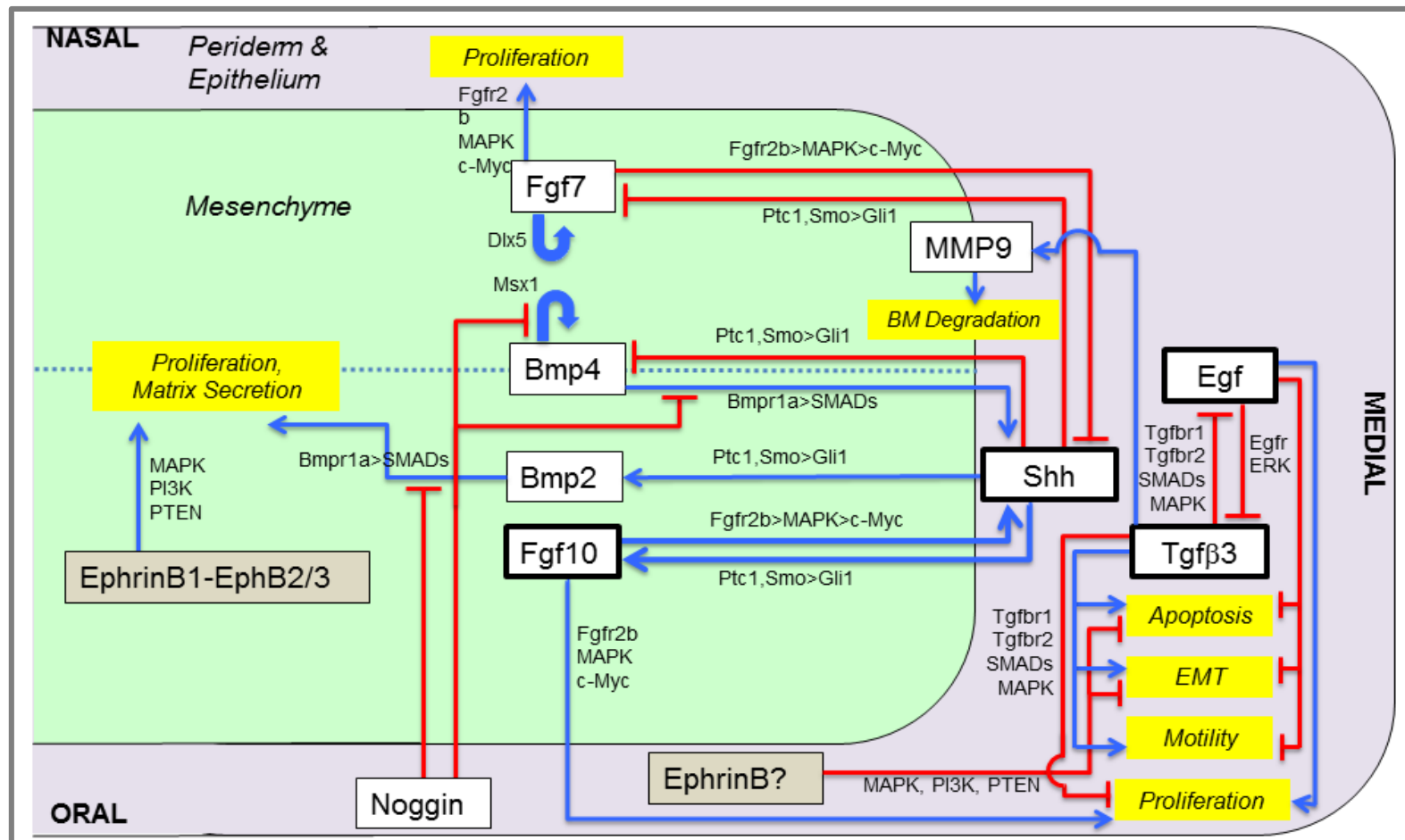
Fusion is delayed by excessive EGF Signaling



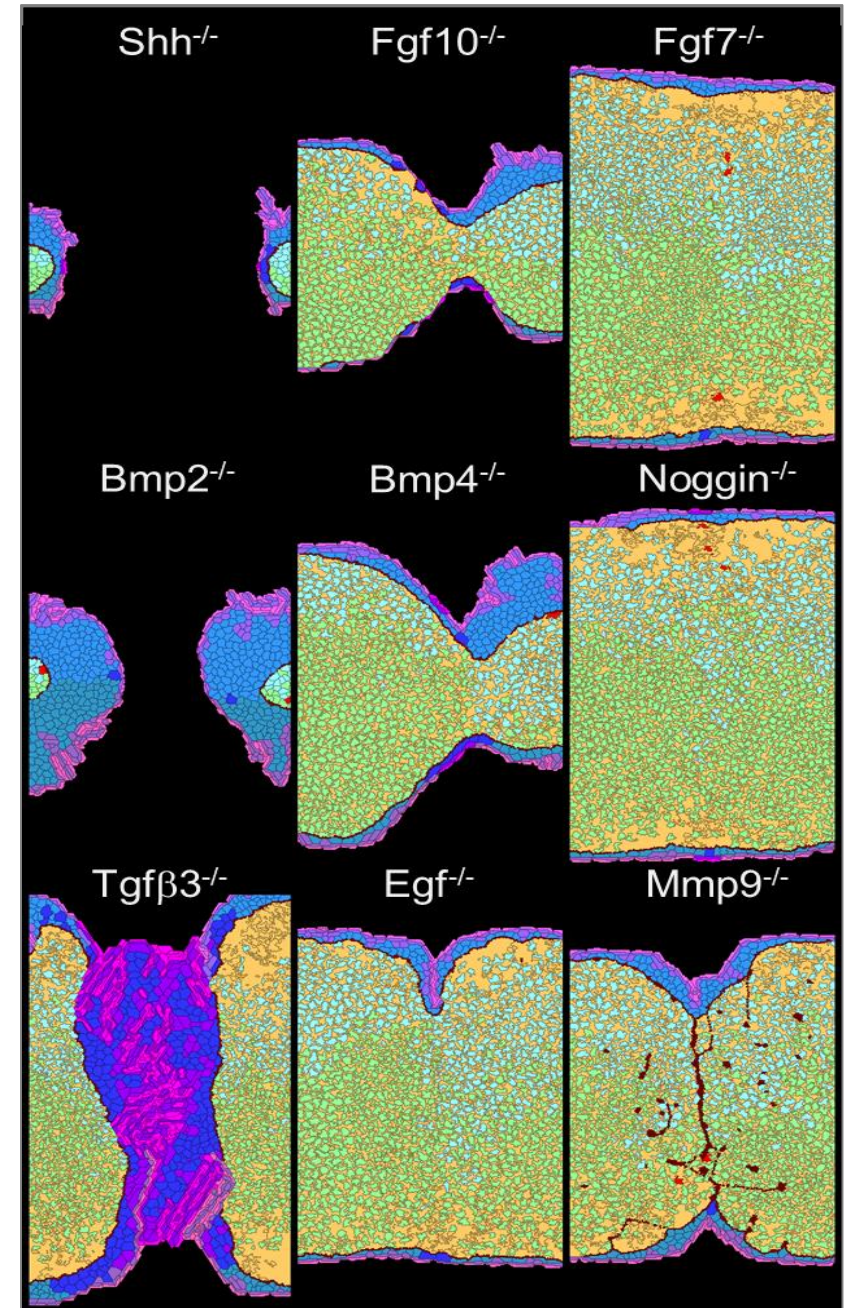
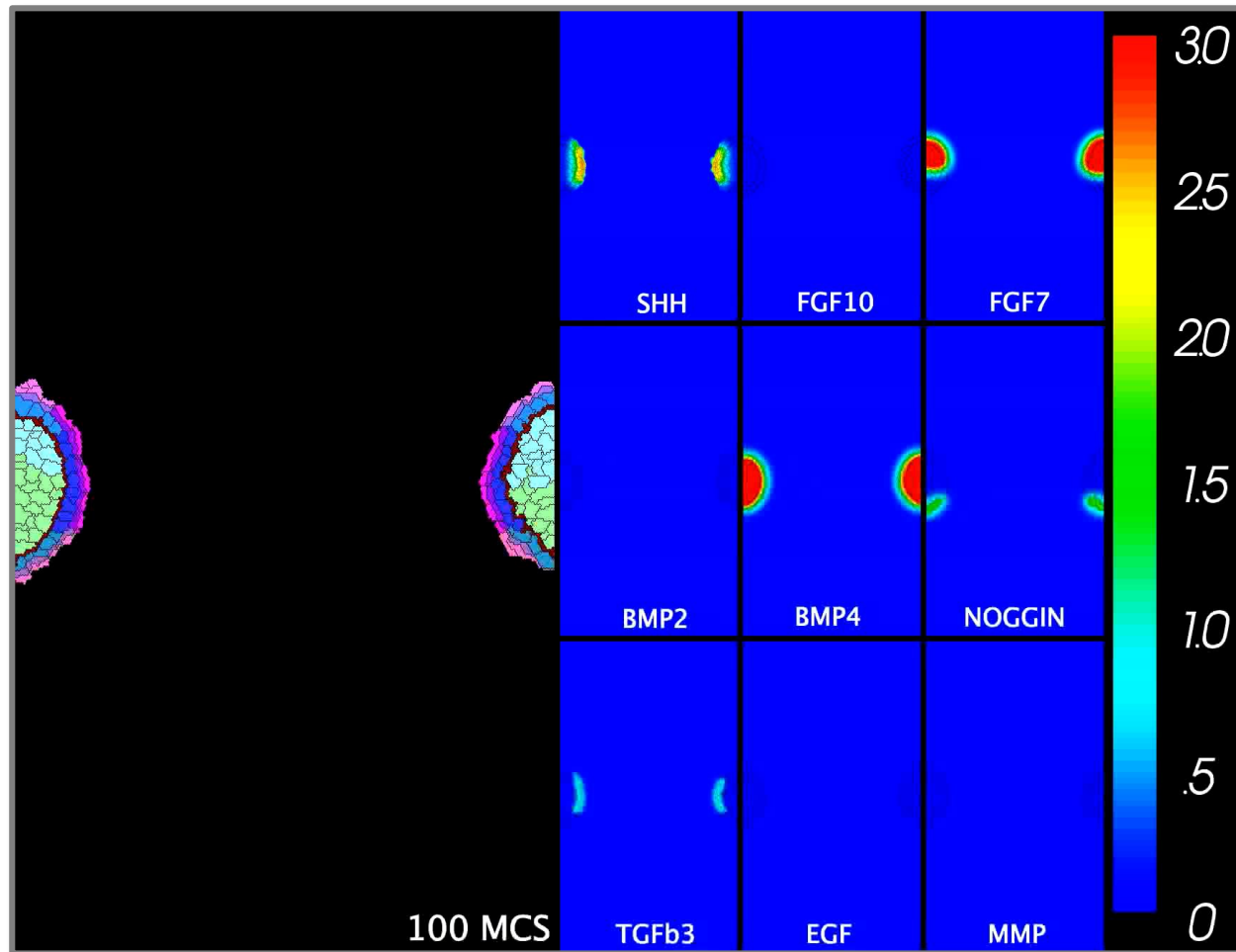
Chemical	Perturbation Days 0-2	Perturbation Days 0-4	Cytotoxicity
TCDD			
All-Trans Retinoic Acid (ATRA)		✓	✓
Dexamethasone			
Corticosterone			
Triamcinolone		✓	
Valproic Acid	✓		
Fluconazole			
Caffeine			
Nicotine			
Tributyltin	✓	✓	✓
Triadimefon			
Theophylline	✓		

- 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.

Systems model: palatal outgrowth and fusion



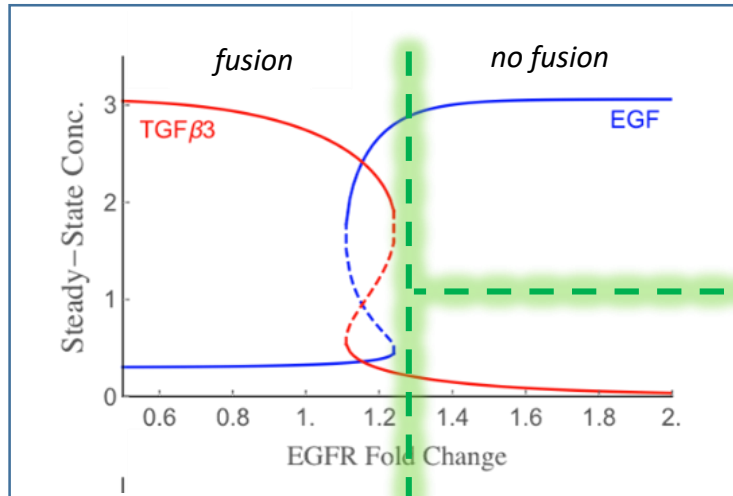
ABM simulation: hacking the control network



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

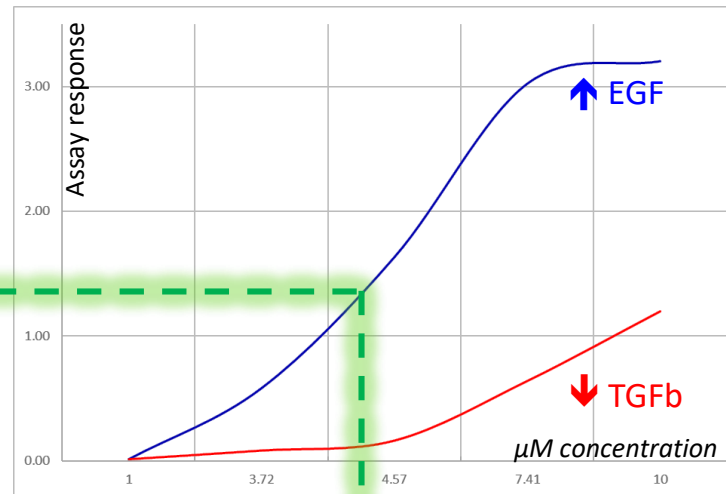
TGF-beta/EGF latch switch: *controls MEE breakdown*

INPUT: switch dynamics



tipping point predicted by
computational dynamics
(hysteresis switch)

Captan in ToxCast

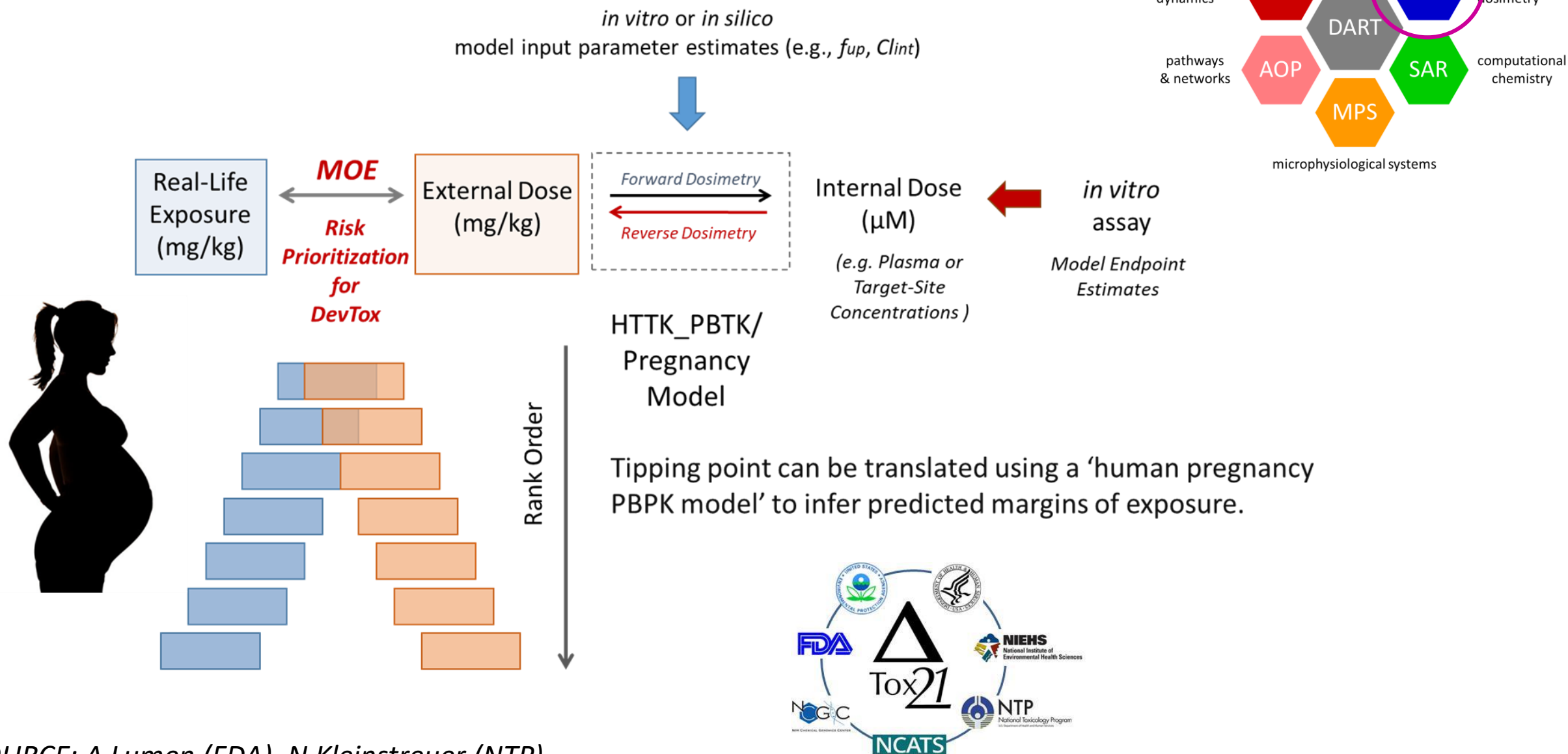


OUTPUT: tipping point
mapped to concentration
response (4 μM)

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

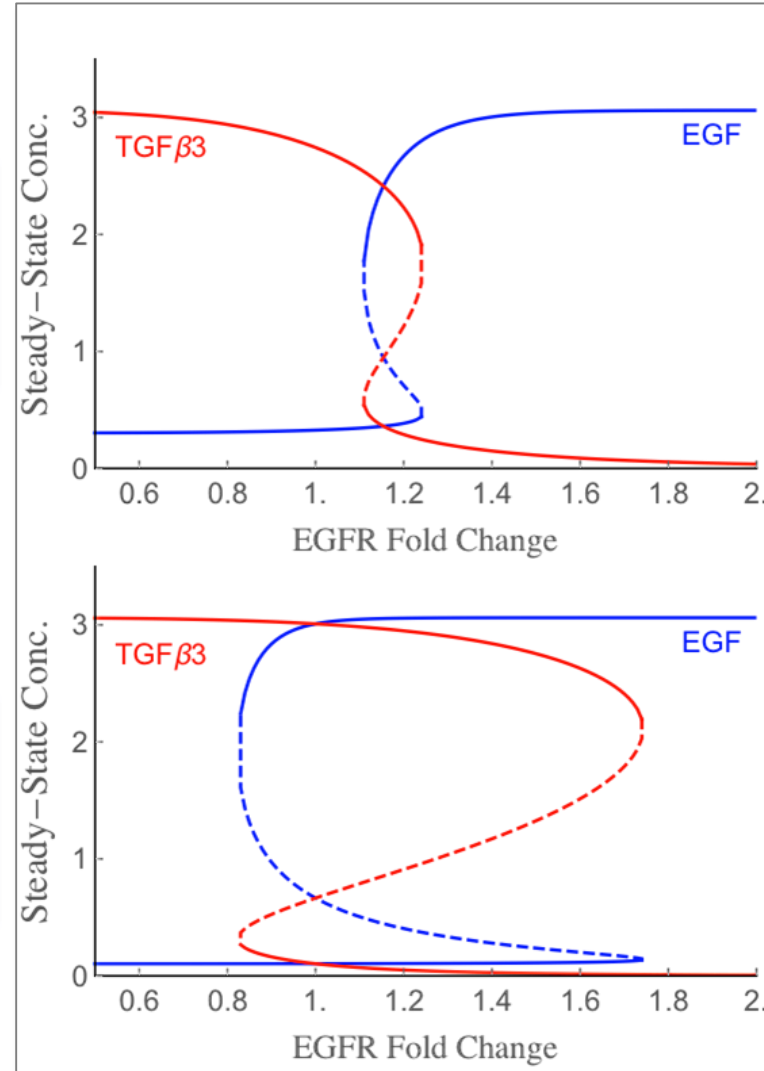
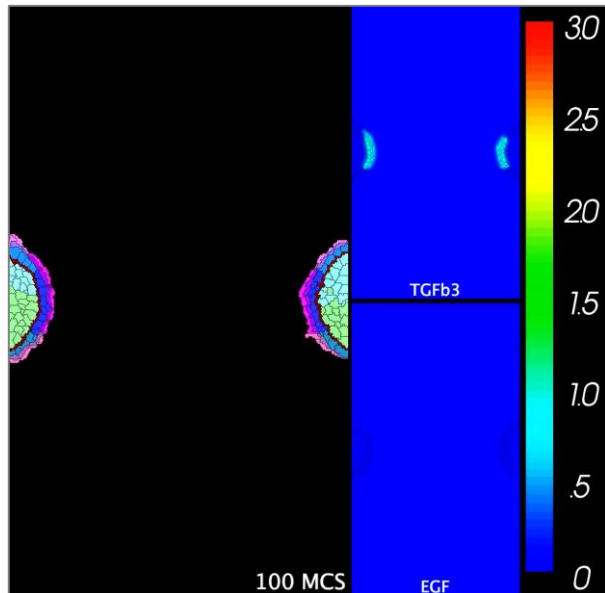
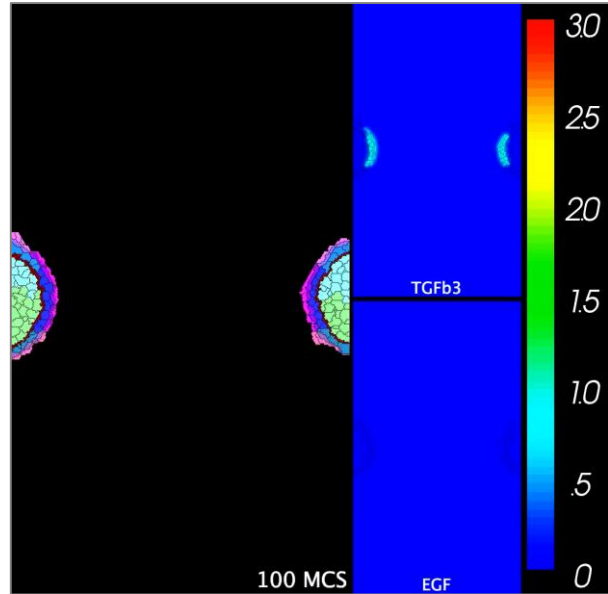
human HTTK model
2.39 mg/kg/day would
achieve a steady state of
4 μM in fetal plasma

httk workflow



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

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



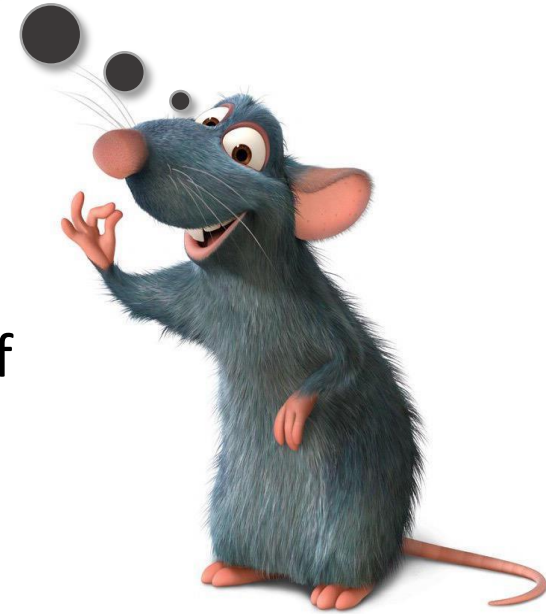
Narrow hysteresis:
less resilient but reversible

Broad hysteresis:
more resilient but irreversible

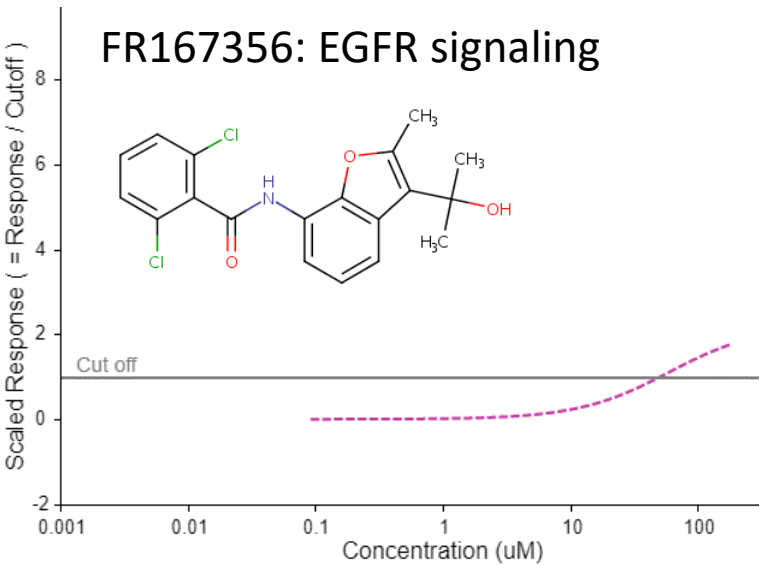
Agent-Based Models (ABMs):

*Multicellular
simulation is 3R's
compliant!*

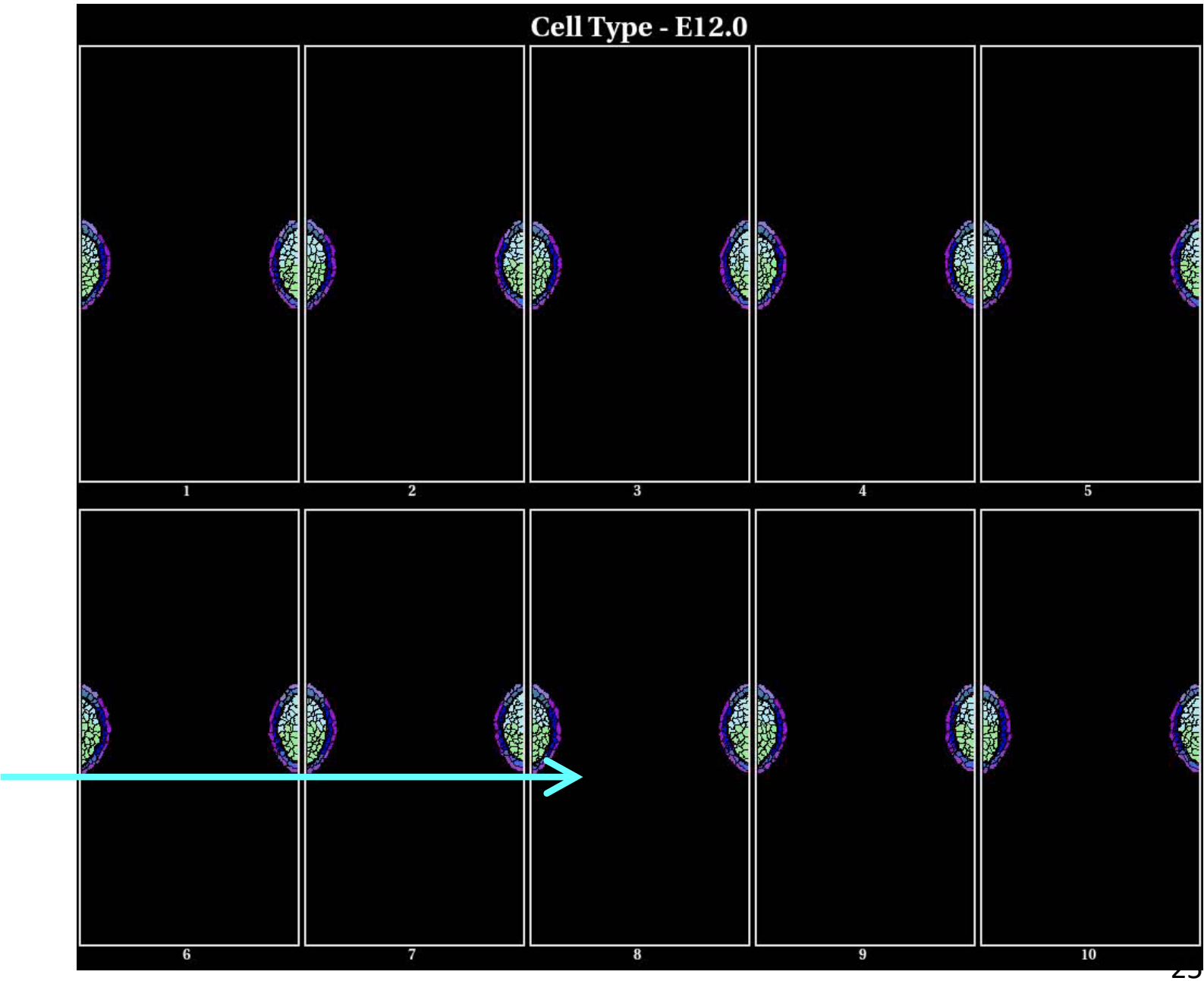
- reconstruct priority systems of embryonic development cell-by-cell and interaction-by-interaction (*emergence*);
- execute tissue simulations that advance through critical determinants of phenotype (*self-organizing phenotypes*);
- simulate *in vitro* data under various *in vivo* scenarios - dose or 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*).



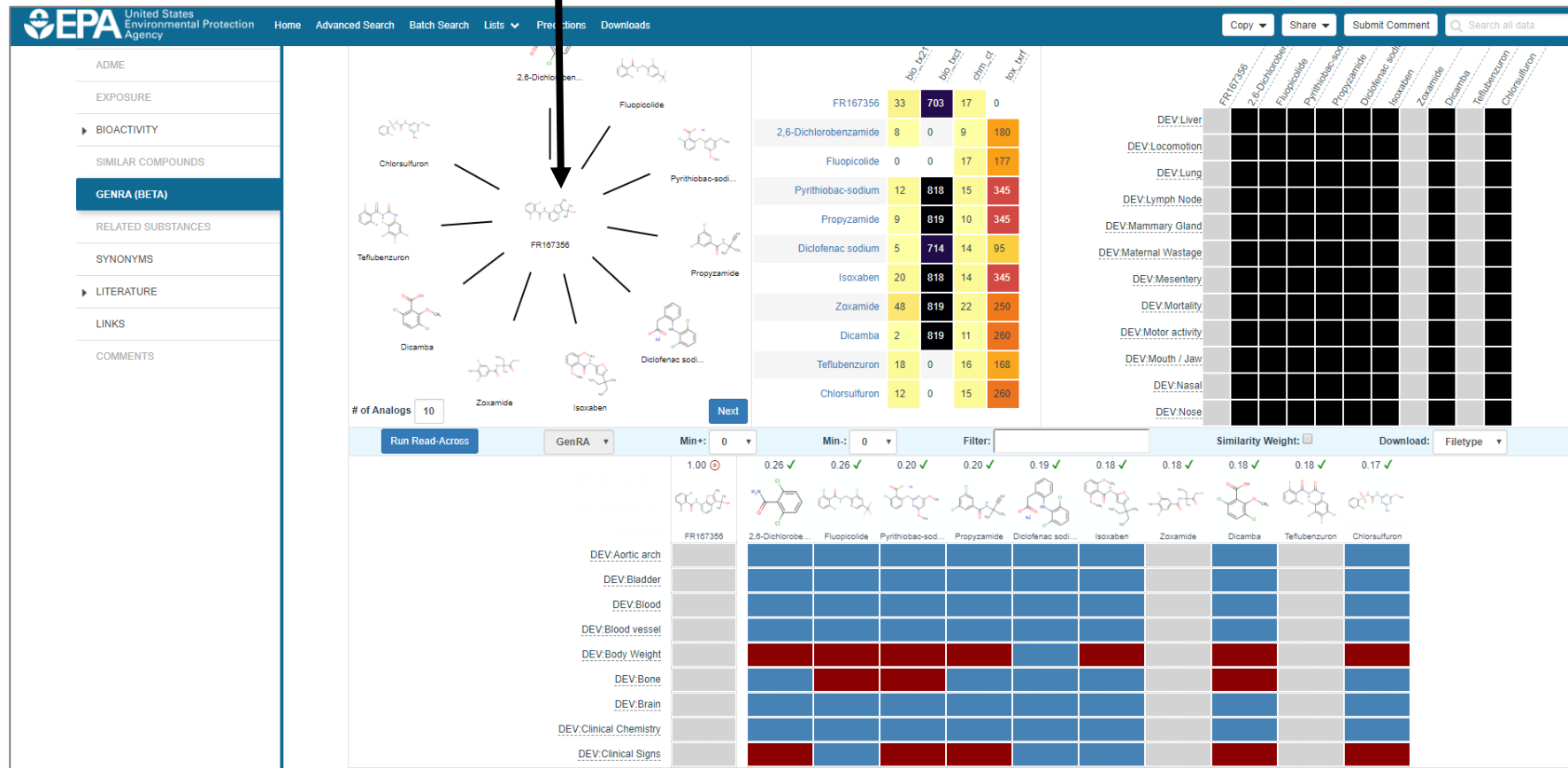
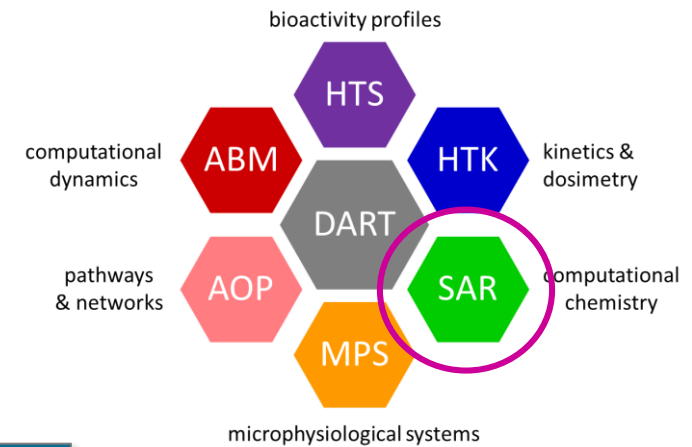
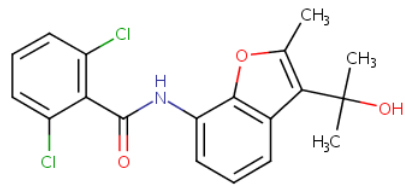
Simulated dose-response



Tipping point predicted
in topological context



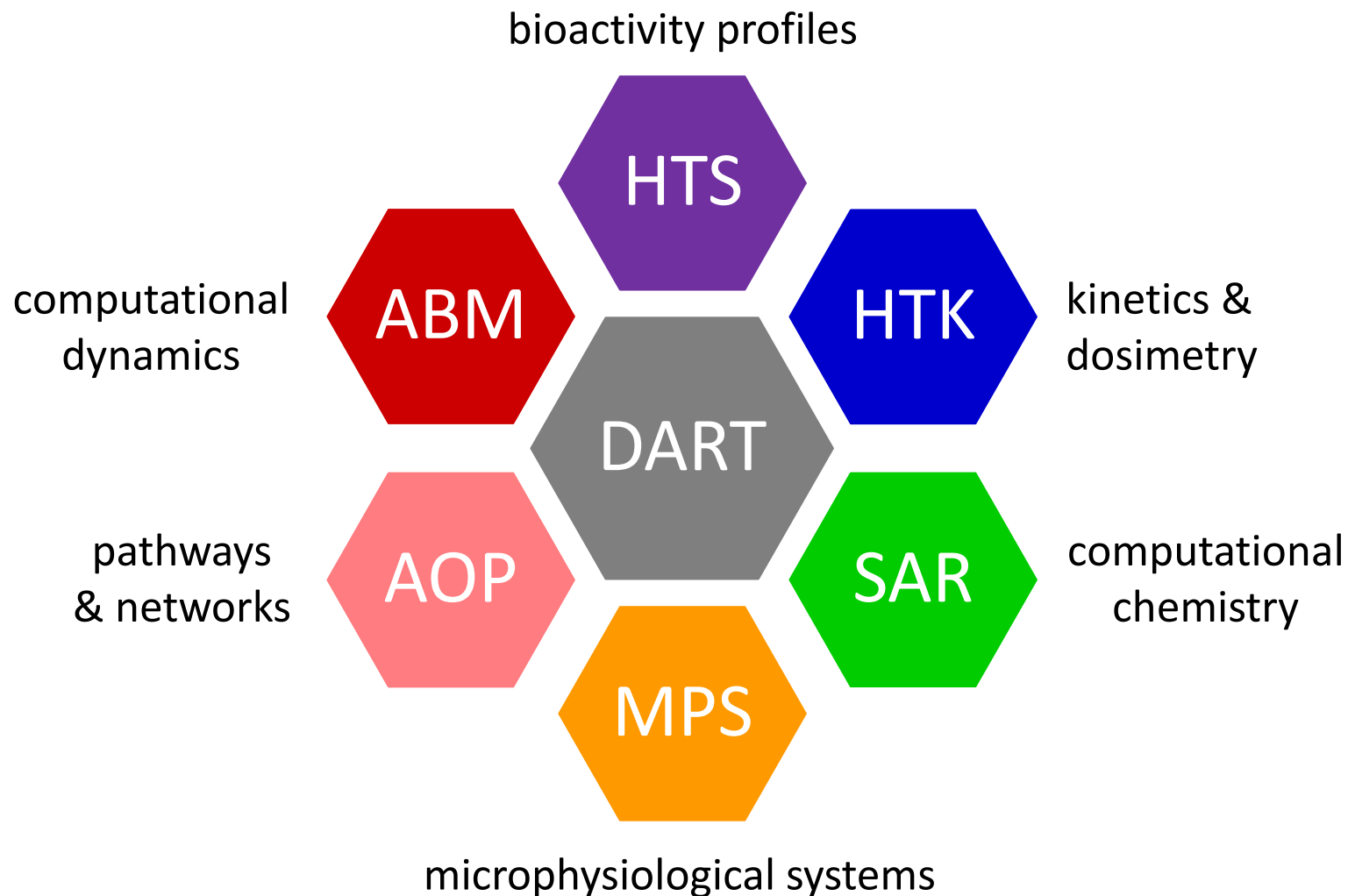
Read across: structural neighbors to the query



- **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							(reproduct ion AND (toxicity OR abnormal OR adverse effects))	nital, Heredit ary, and Neonat al Disease s and Abnorm alities[	palate OR palate/ drug effects) not surgery AND develop ment	
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-Dichlorobenzam	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	Pyriethiobac-sodium	123343-16-8 OR Pyriethiobac-sodium OR pyriethiobac 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										

IATA: computational synthesis and integration



Special Thanks



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Nathan Rush - NCCT
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Rusty Thomas – NCCT
Todd Zurlinden – NCCT



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Jessica Palmer - Stemina



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