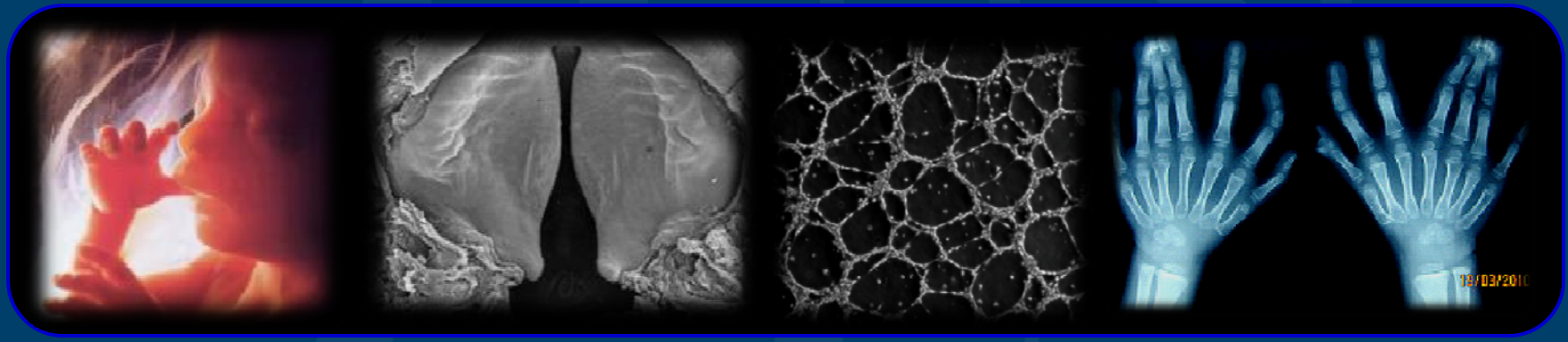
EPA
United States
Environmental Protection
Agency

Revolutionizing Toxicity Testing for Predicting Developmental Outcomes

Nisha S. Sipes, PhD
ORISE Fellow
National Center for Computational Toxicology, USEPA



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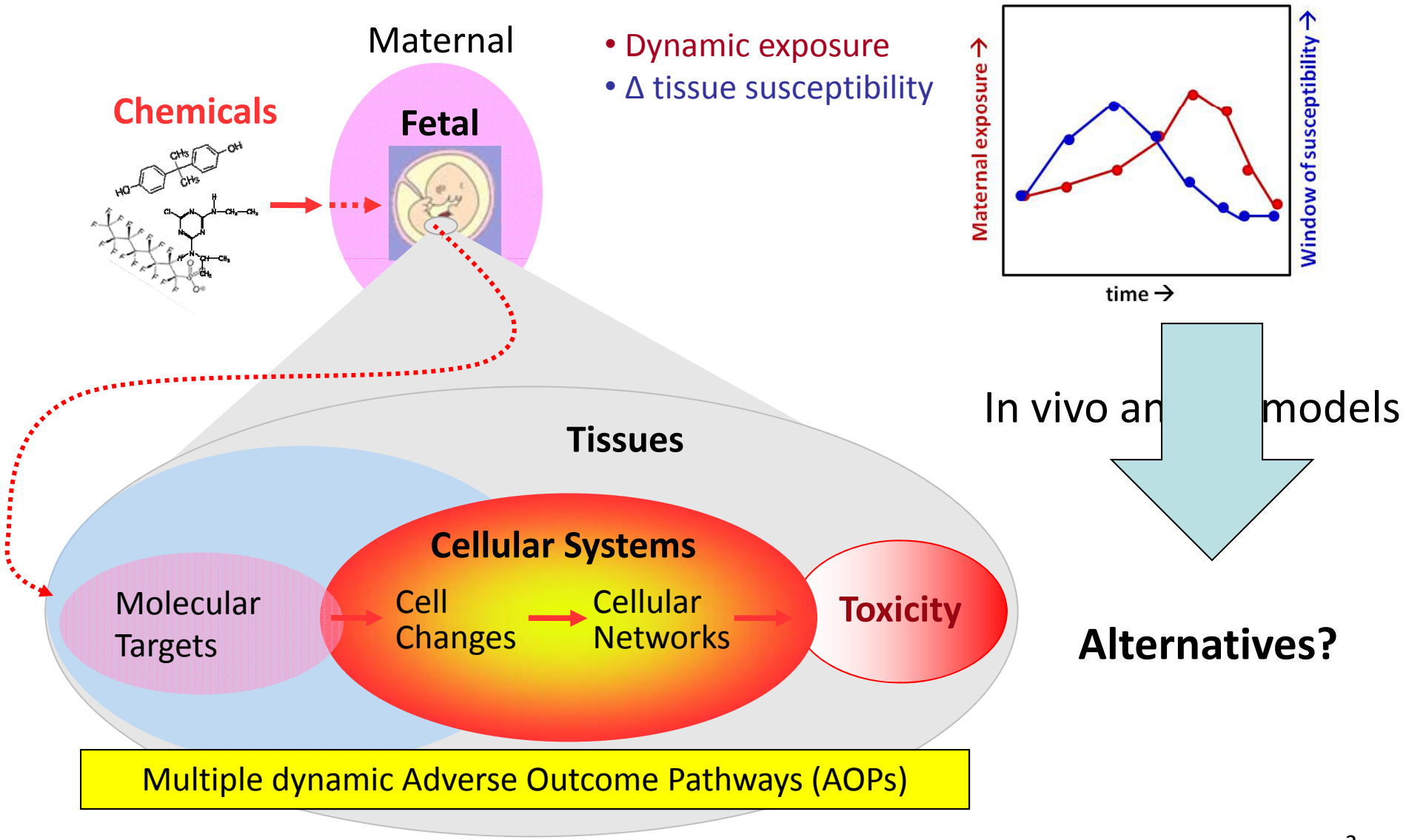
Office of Research and Development
National Center for Computational Toxicology
www.epa.gov/ncct

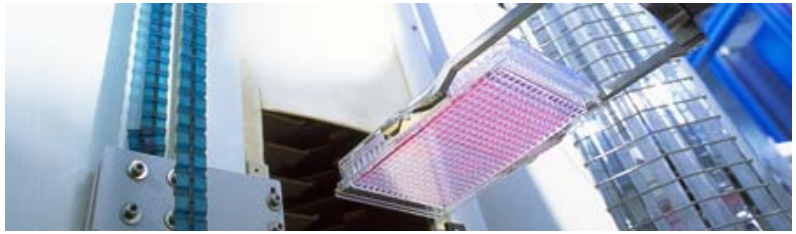
Fourth International Conference on Alternatives for Developmental Neurotoxicity (DNT) Testing, May 14, 2014

Predicting Developmental Toxicity



Computational Toxicology:

high-throughput screening (HTS)



ToxCast: EPA research **http://www.epa.gov/ncct/toxcast/**

>1060 chemicals

>700 *in vitro* assays (27M data points)

Biological Response
cell proliferation and death
cell differentiation
enzymatic activity
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA)
receptor binding
receptor activity
steroidogenesis

Target Family
response Element
transporter
cytokines
kinases
nuclear receptor
CYP450 / ADME
cholinesterase
phosphatases
proteases
XME metabolism
GPCRs
ion channels

DevTox (new)
ES cell secretome
neuroprogenitors
morphogenesis
differentiation

Assay Design
viability reporter
morphology reporter
conformation reporter
enzyme reporter
mem. potential reporter
binding reporter
inducible reporter

Cell Format
cell free
cell lines
primary cells
complex cultures
free embryos

Tissue Source

Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

Detection Technology
qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrayscan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

Tox21: partnership of federal agencies

~10,000 chemicals in dozens of HTS assays

3

Computational Toxicology:

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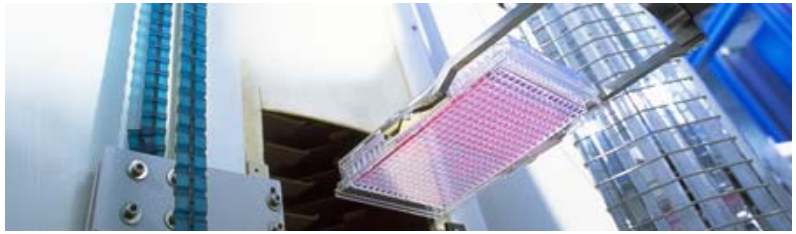

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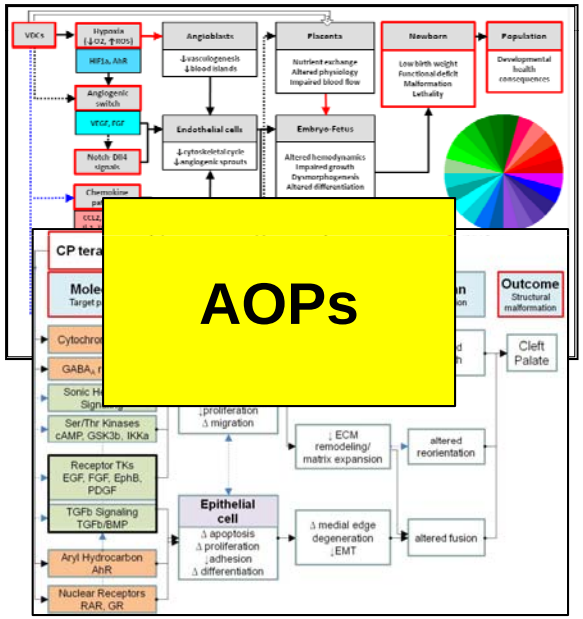
Predictive Signatures



AOPs

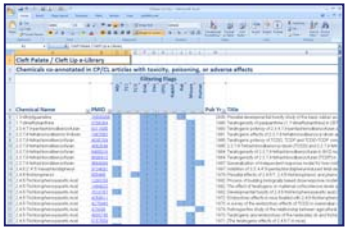
Prioritization

Hypothesis generation

Cleft Palate AOP Framework

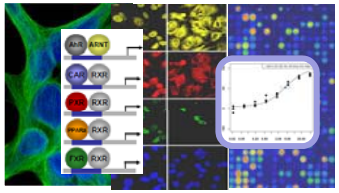
CP teratogens

Molecular	Cellular	Tissue	Organ	Outcome
Target pathways	Cell-level behaviors	Palatal shelf	Apposition	Structural malformation



1. e-libraries

2.informatics.jax.org



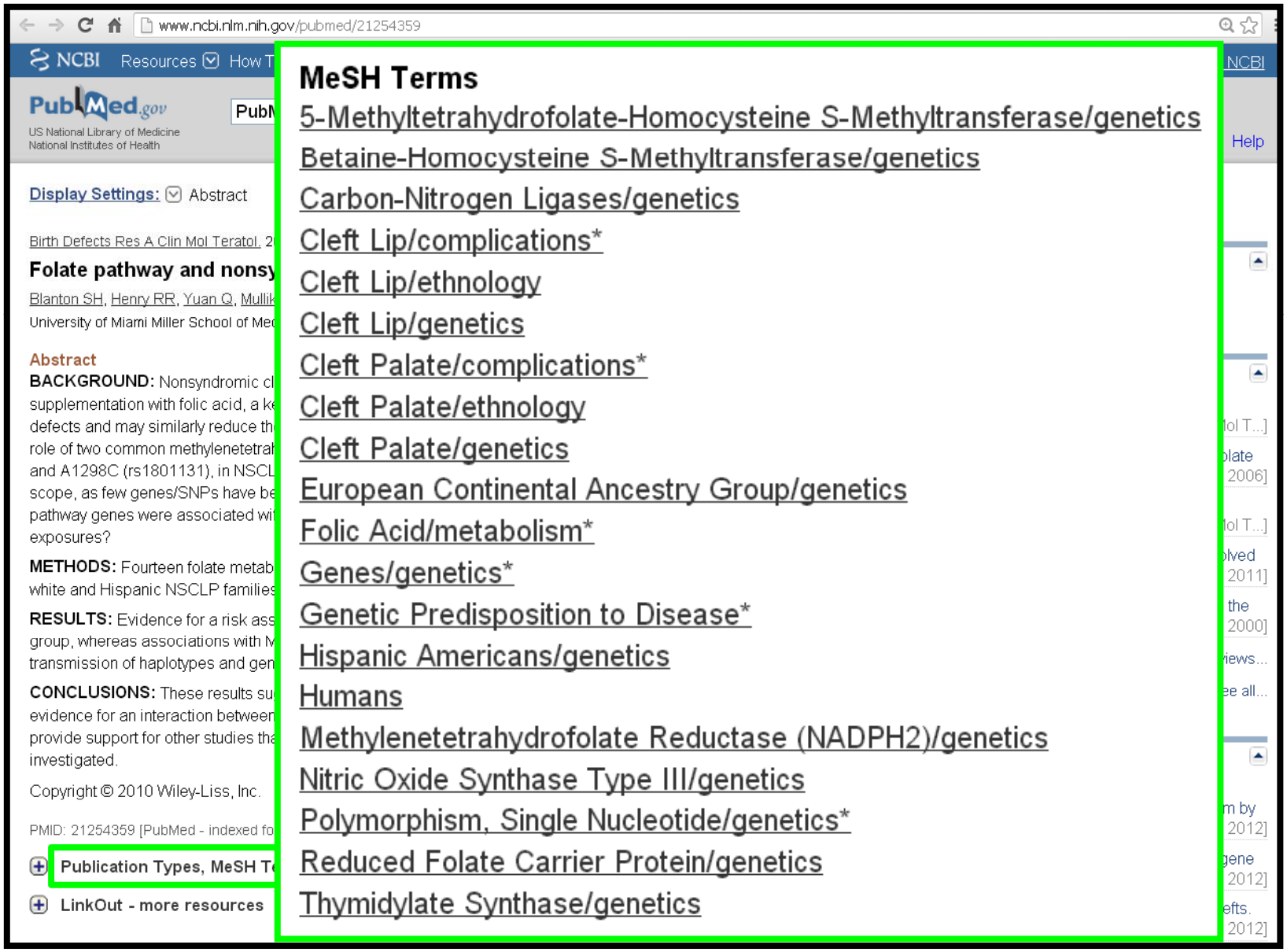
3. ToxCast

- altered growth
- altered reorientation
- altered fusion

→ Cleft Palate

1. e-library Development

Literature Mining Using MeSH Annotations



1. e-library Development

>20,000 Cleft Lip/Cleft Palate Articles

Query within Cleft Lip/Palate Articles using MeSH Dictionaries

Proteins:	1249 unique
Protein-protein:	5532 unique
Cell processes:	92 unique
Chemicals:	1009 unique

- 481 unique (toxicity poisoning, adverse effects)

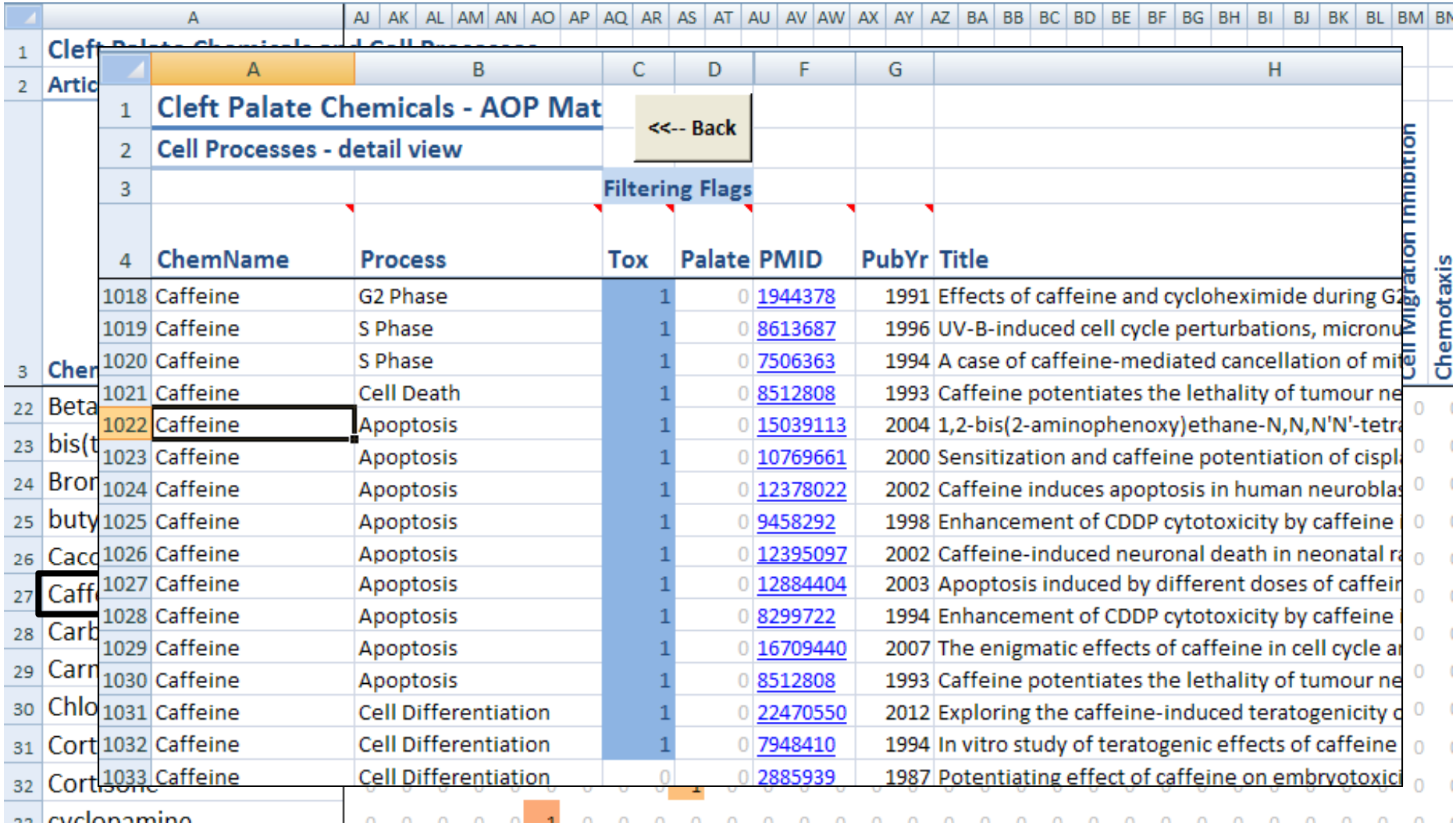
1. e-library Development

CLP Chemicals and Cell Processes

	A	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
1	Cleft Palate Chemicals and Cell Processes																															
2	Article counts for co-occurring MeSH terms annotated with drug effects																															
3	Chemicals	G0 Phase	G1 Phase	G2 Phase	S Phase	Cell Death	Apoptosis	Anoikis	Autophagy	Cell Dedifferentiation	Cell Differentiation	Adipogenesis	Gametogenesis	Oogenesis	Spermatogenesis	Sperm Maturation	Hematopoiesis	Erythropoiesis	Hematopoiesis, Extramedi	Leukopoiesis	Lymphopoiesis	Myelopoiesis	Neurogenesis	Cell Fusion	Cell Growth Processes	Cell Enlargement	Cell Proliferation	Cell Division	Cell Movement	Cell Aggregation	Cell Migration Inhibition	Chemotaxis
22	Betamethasone	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	
23	bis(tri-n-butyltin)oxide	0	0	0	0	0	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	
24	Bromodeoxyuridine	1	1	1	3	10	10	0	0	0	14	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	17	28	4	0	0	
25	butylbenzyl phthalate	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	
26	Cacodylic Acid	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	6	0	0	0	
27	Caffeine	0	0	7	2	1	9	0	0	0	5	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	2	13	1	1	0	
28	Carbaryl	0	0	0	0	1	0	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	
29	Carmustine	0	0	2	0	2	3	0	0	0	1	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	1	15	1	0	0	
30	Chlorpyrifos	0	0	0	0	6	10	0	0	0	14	1	1	2	2	0	0	0	0	0	0	0	4	0	0	0	4	4	1	0	0	
31	Corticosterone	0	0	0	0	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	
32	Cortisone	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	cyclopamine	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

1. e-library Development

CLP Chemicals and Cell Processes



Cleft Palate Chemicals - AOP Mat

<<-- Back

Cell Processes - detail view

Filtering Flags

	ChemName	Process	Tox	Palate	PMID	PubYr	Title
1018	Caffeine	G2 Phase	1	0	1944378	1991	Effects of caffeine and cycloheximide during G2
1019	Caffeine	S Phase	1	0	8613687	1996	UV-B-induced cell cycle perturbations, micronu
1020	Caffeine	S Phase	1	0	7506363	1994	A case of caffeine-mediated cancellation of mit
1021	Caffeine	Cell Death	1	0	8512808	1993	Caffeine potentiates the lethality of tumour ne
1022	Caffeine	Apoptosis	1	0	15039113	2004	1,2-bis(2-aminophenoxy)ethane-N,N,N'N'-tetra
1023	Caffeine	Apoptosis	1	0	10769661	2000	Sensitization and caffeine potentiation of cispl
1024	Caffeine	Apoptosis	1	0	12378022	2002	Caffeine induces apoptosis in human neurobla
1025	Caffeine	Apoptosis	1	0	9458292	1998	Enhancement of CDDP cytotoxicity by caffeine
1026	Caffeine	Apoptosis	1	0	12395097	2002	Caffeine-induced neuronal death in neonatal ra
1027	Caffeine	Apoptosis	1	0	12884404	2003	Apoptosis induced by different doses of caffein
1028	Caffeine	Apoptosis	1	0	8299722	1994	Enhancement of CDDP cytotoxicity by caffeine
1029	Caffeine	Apoptosis	1	0	16709440	2007	The enigmatic effects of caffeine in cell cycle ar
1030	Caffeine	Apoptosis	1	0	8512808	1993	Caffeine potentiates the lethality of tumour ne
1031	Caffeine	Cell Differentiation	1	0	22470550	2012	Exploring the caffeine-induced teratogenicity c
1032	Caffeine	Cell Differentiation	1	0	7948410	1994	In vitro study of teratogenic effects of caffeine
1033	Caffeine	Cell Differentiation	0	0	2885939	1987	Potentiating effect of caffeine on embryotoxici

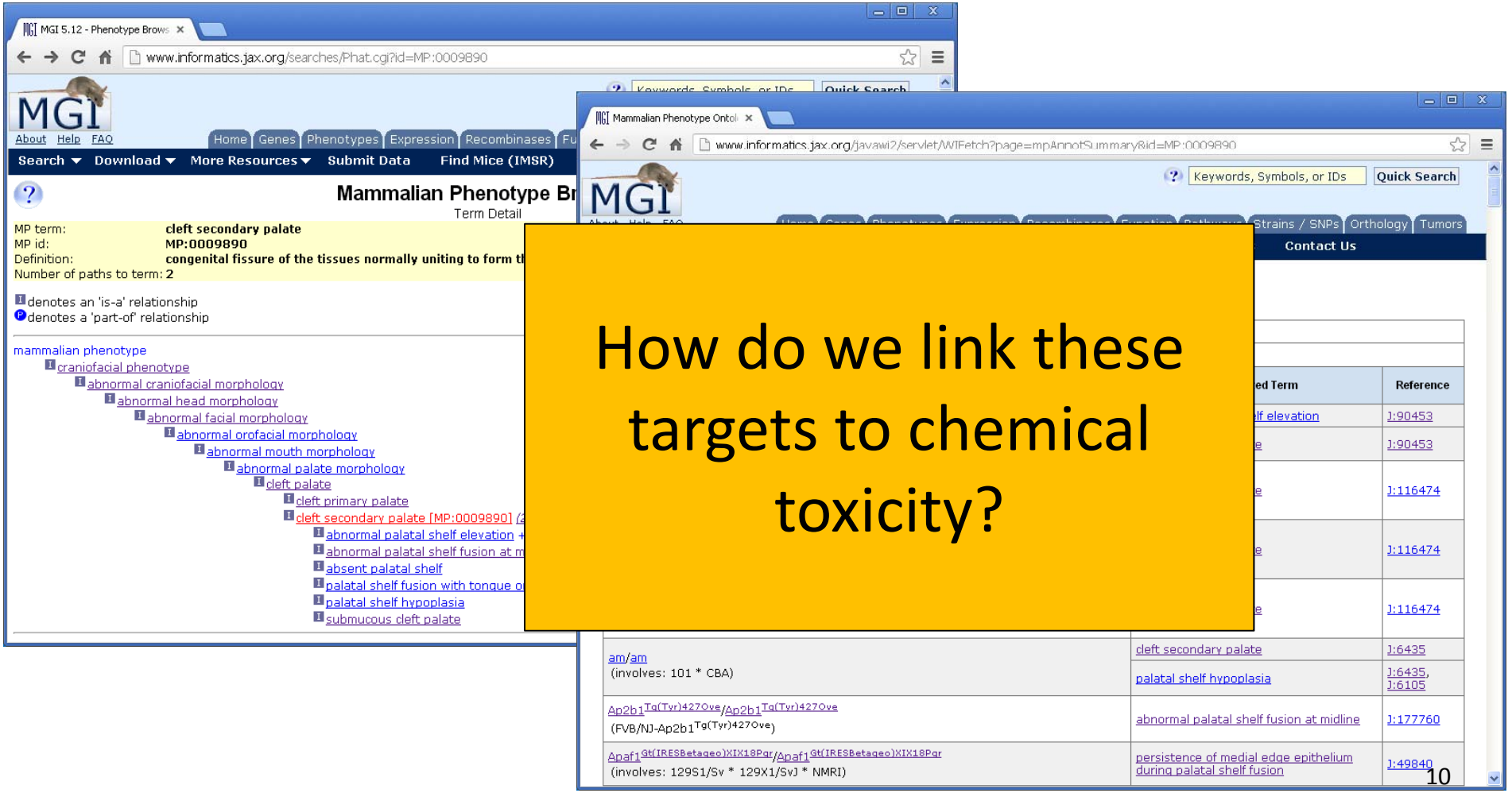
Cell Migration Inhibition

Chemotaxis

2. informatics.jax.org

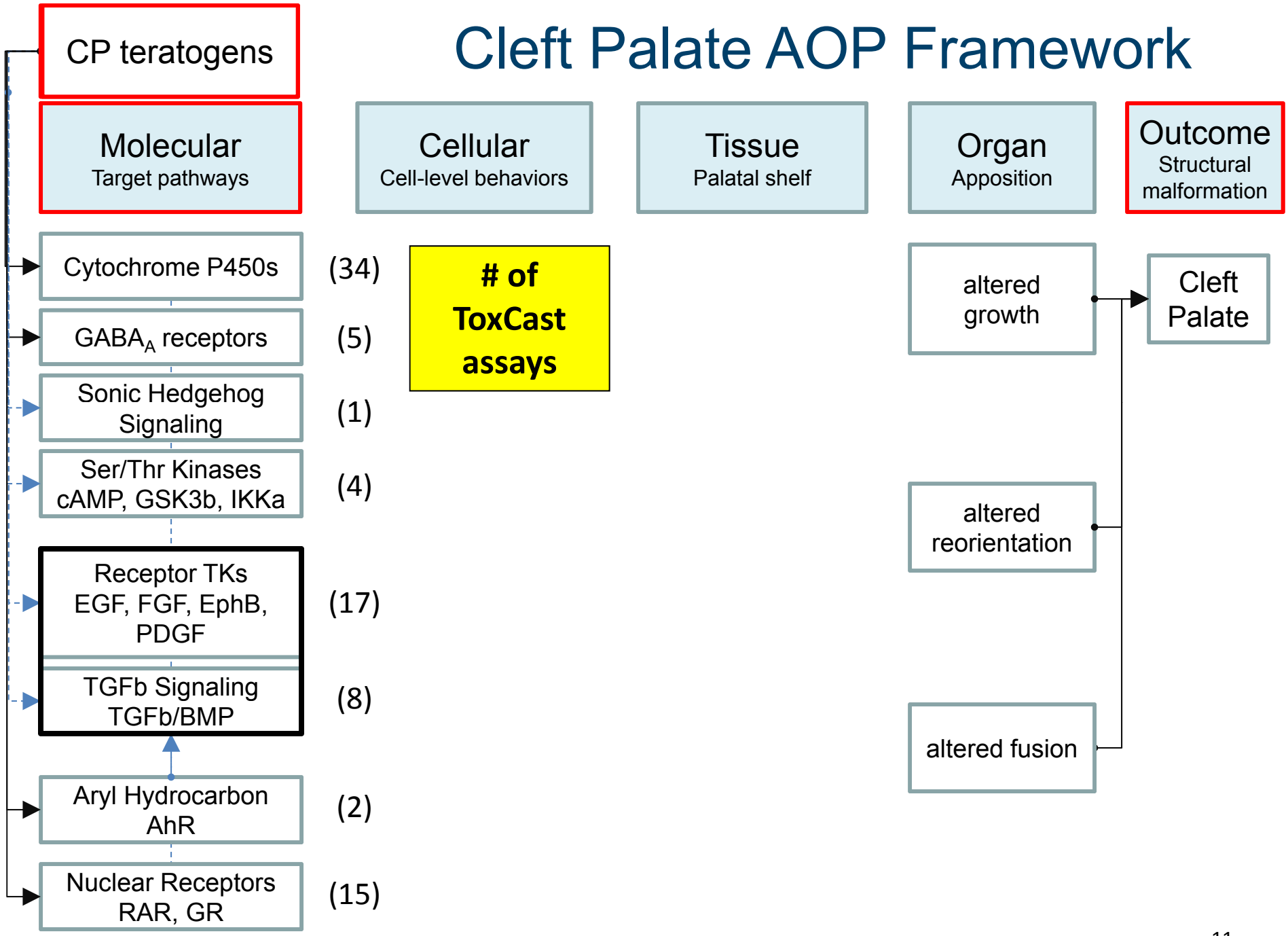
cleft secondary palate [MP:0009890] (231 genotypes, 351 annotations)

- ~111 genes

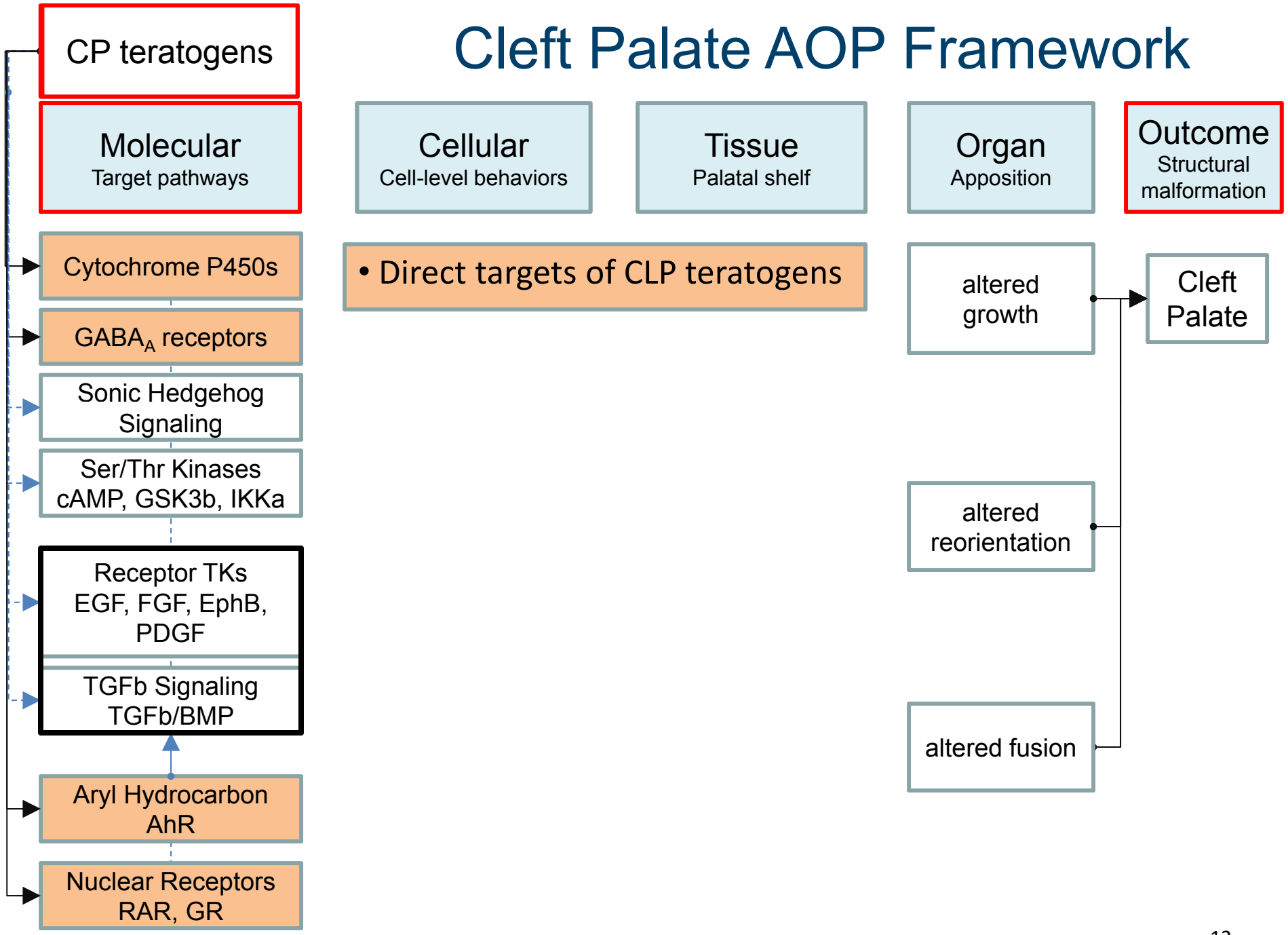


How do we link these targets to chemical toxicity?

Cleft Palate AOP Framework



Cleft Palate AOP Framework



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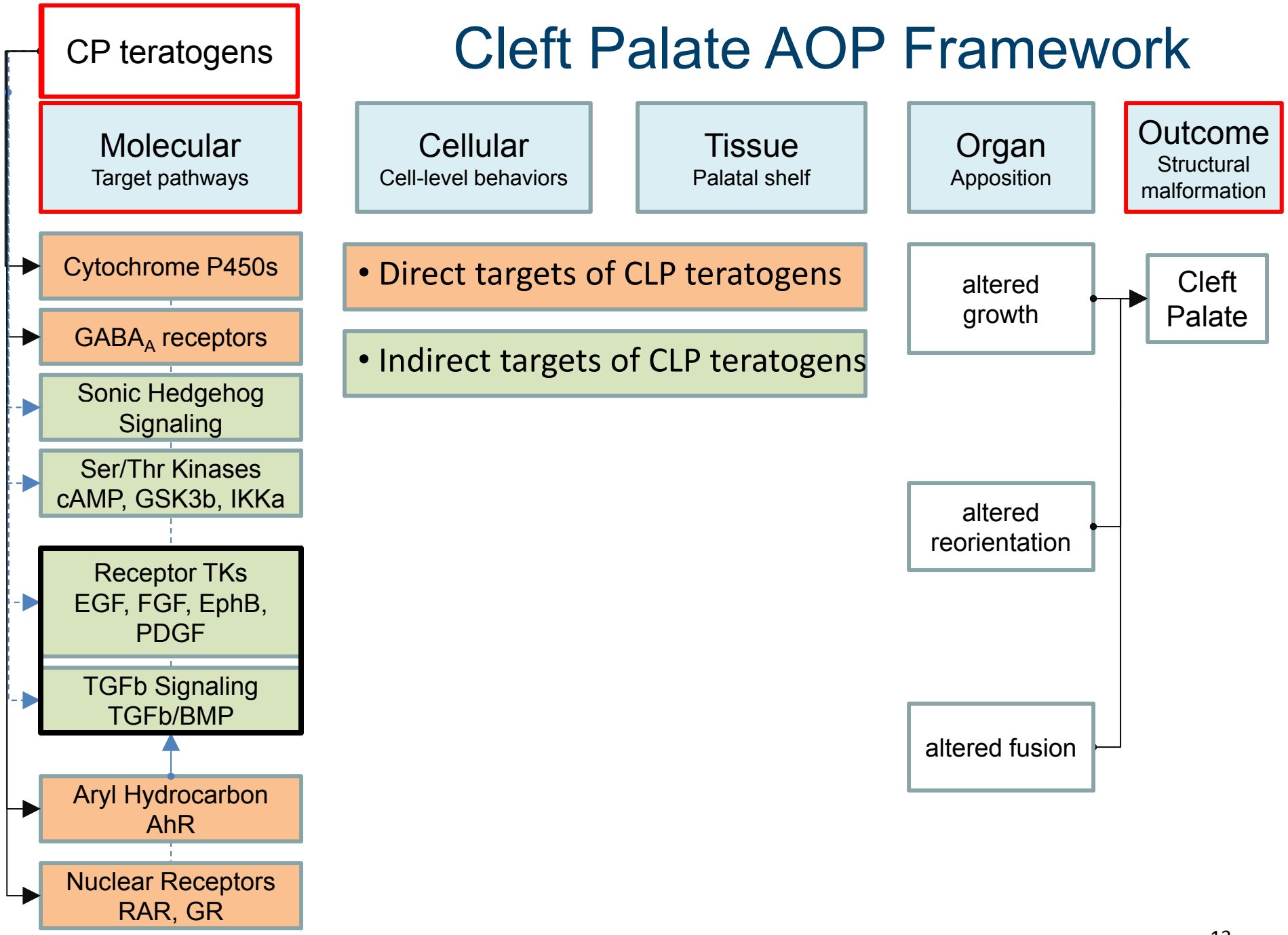


Cleft Palate AOP Framework

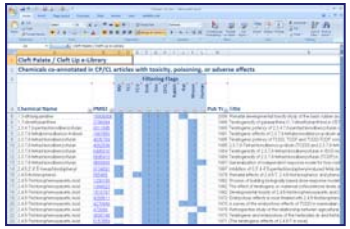
CP teratogens

Molecular
Target pathways

- Cytochrome P450s
- $GABA_A$ receptors
- Sonic Hedgehog Signaling
- Ser/Thr Kinases cAMP, GSK3b, IKKa
- Receptor TKs EGF, FGF, EphB, PDGF
- TGFb Signaling TGFb/BMP
- Aryl Hydrocarbon AhR
- Nuclear Receptors RAR, GR

Cellular
Cell-level behaviors

Tissue
Palatal shelf



e-libraries

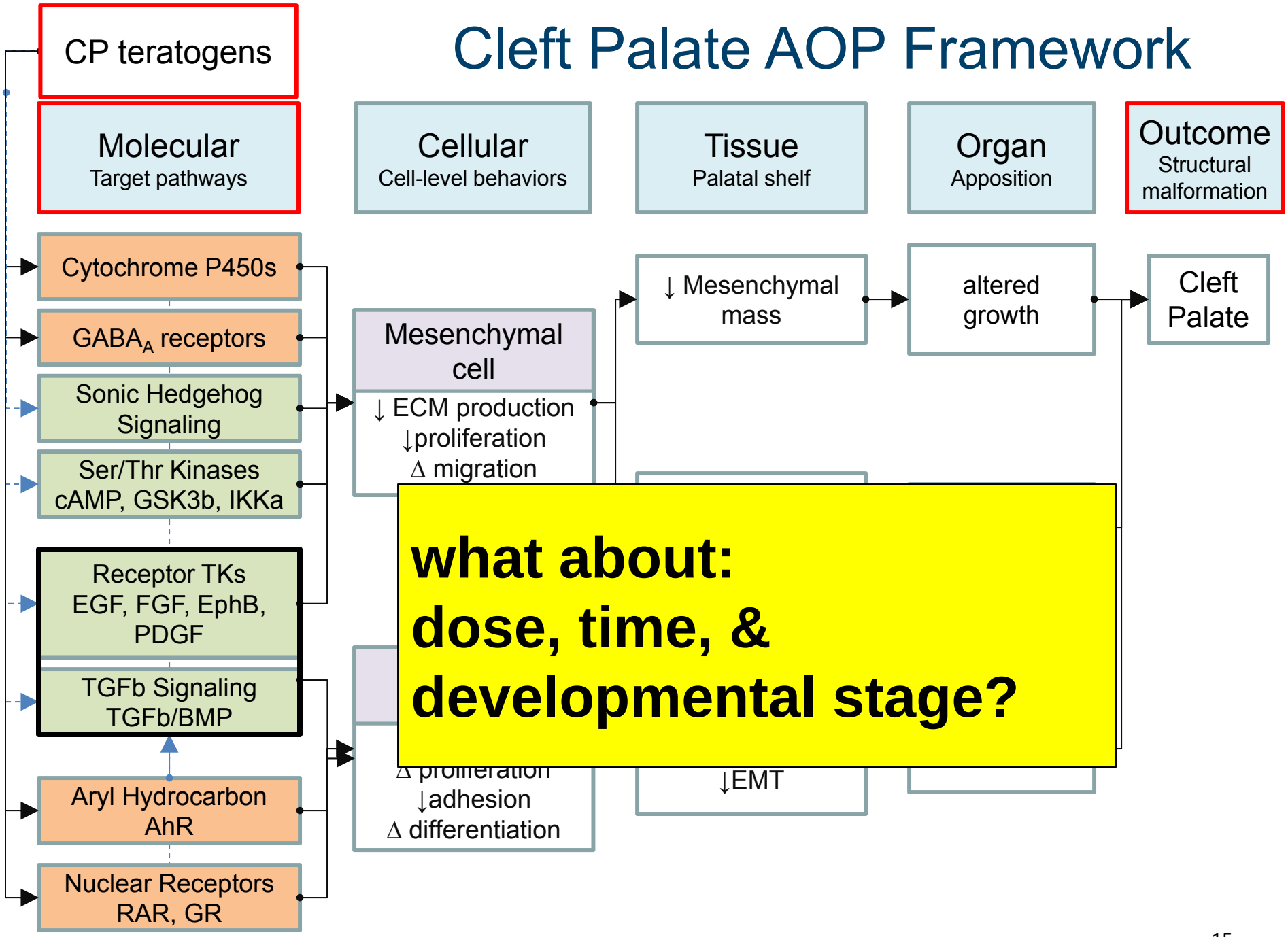
Organ
Apposition

- altered growth
- altered reorientation
- altered fusion

Outcome
Structural malformation

- Cleft Palate

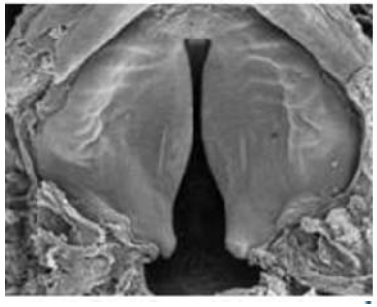
Cleft Palate AOP Framework

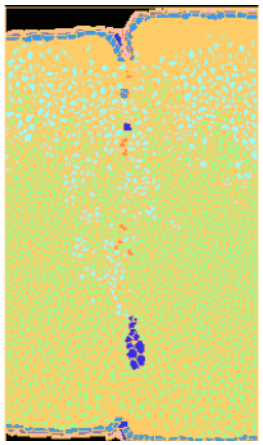


Multicellular Virtual-Tissue Models (VTMs)

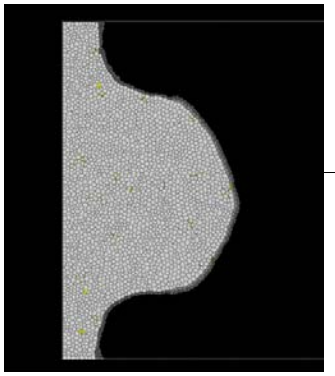
Cell-level models driven by biological networks and rules

Palate

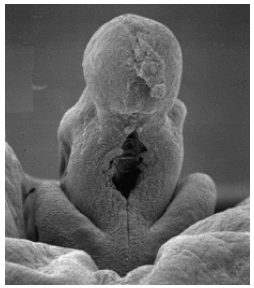




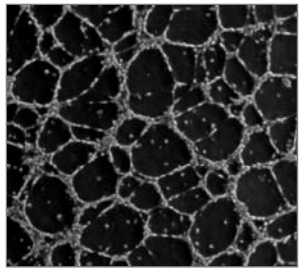
Limb-bud

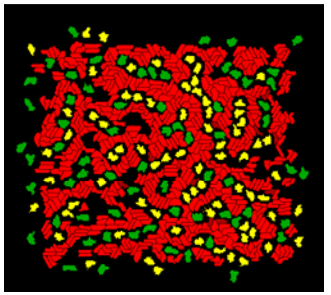


Genital tubercle



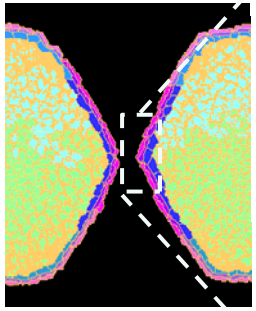
Blood Vessel

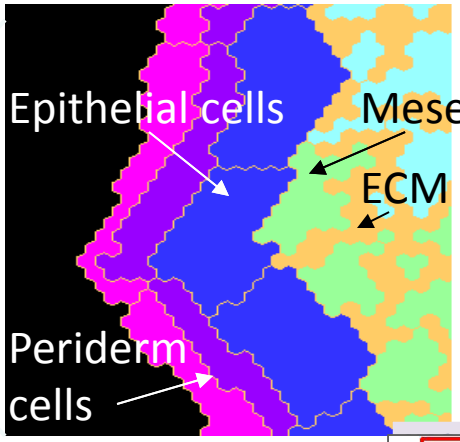




Cell *Agent-Based Models* (ABMs)

Palatal fusion



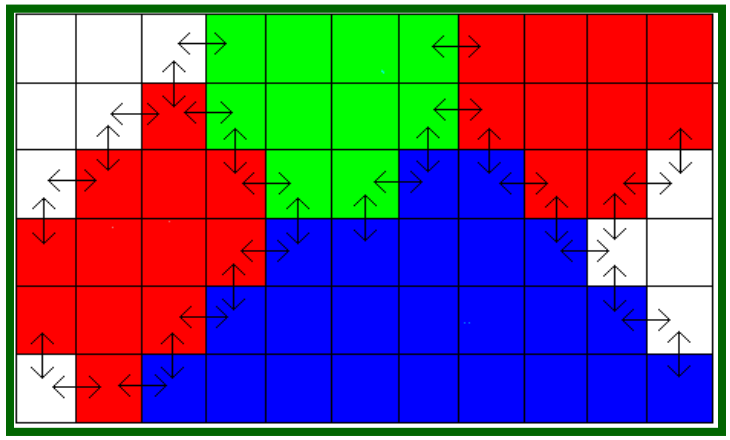


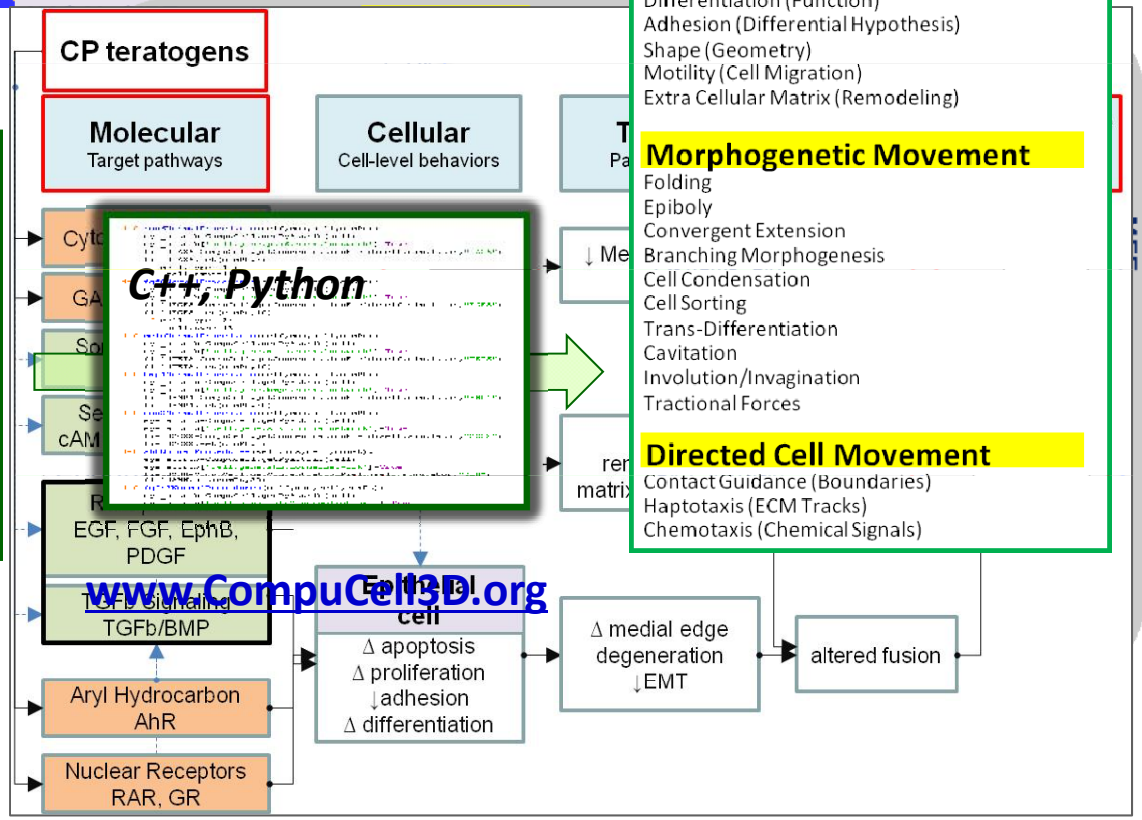
Core Developmental Processes
Patterning (Sets up Future Events)
Timing (Clocks and Oscillators)
Differentiation (Cell Diversification)
Morphogenesis (Tissue Organization)

Cellular Primitives
Growth (Proliferation)
Growth (Volume Increase)
Death (Apoptosis)
Differentiation (Function)
Adhesion (Differential Hypothesis)
Shape (Geometry)
Motility (Cell Migration)
Extra Cellular Matrix (Remodeling)

Morphogenetic Movement
Folding
Epiboly
Convergent Extension
Branching Morphogenesis
Cell Condensation
Cell Sorting
Trans-Differentiation
Cavitation
Involution/Invagination
Tractional Forces

Directed Cell Movement
Contact Guidance (Boundaries)
Haptotaxis (ECM Tracks)
Chemotaxis (Chemical Signals)





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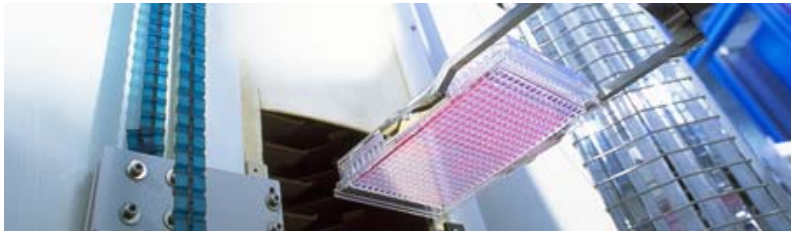
Computational Toxicology:

high-throughput screening (HTS)



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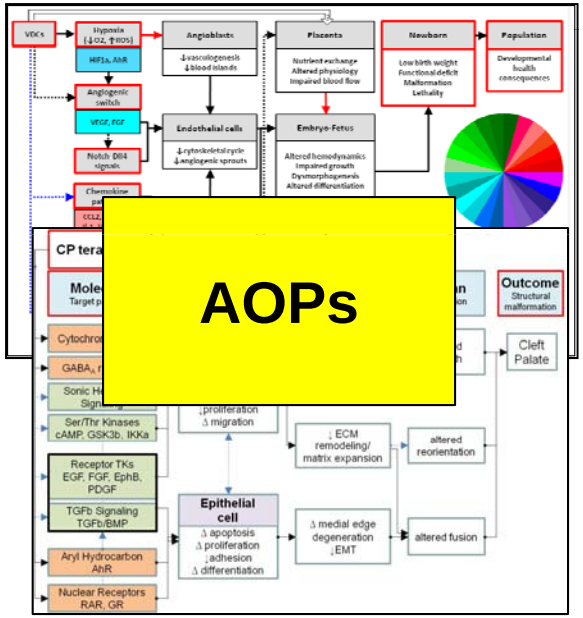

AOPs

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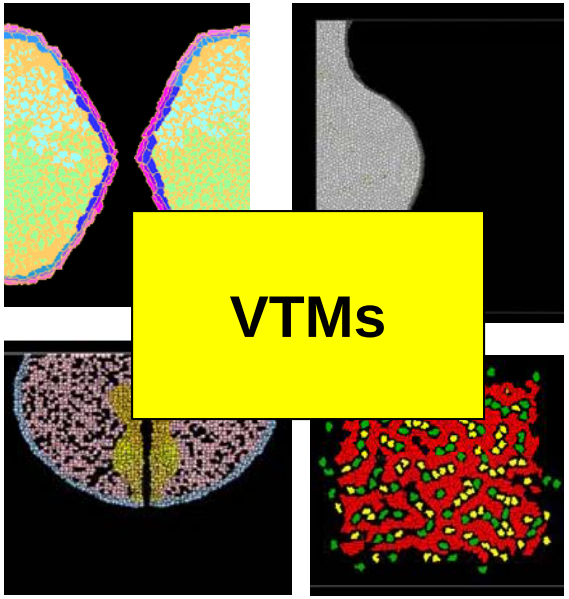
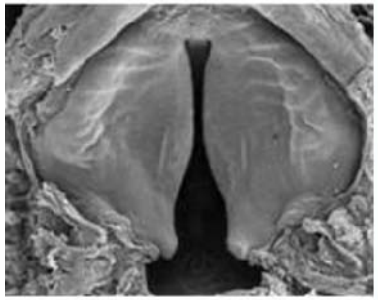


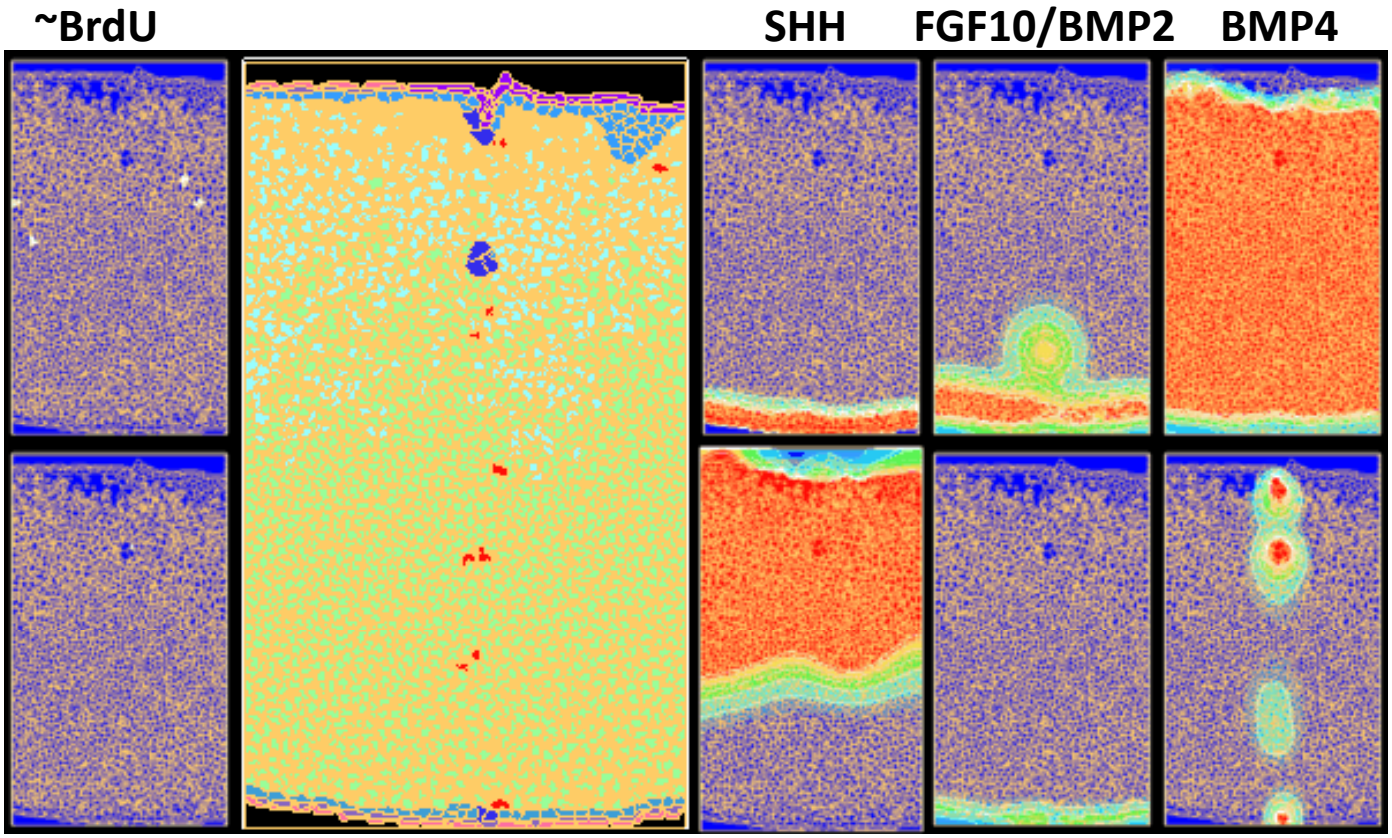
VTMs

Prediction

multiple AOPs, dose, mixtures, time, stage, plausible 'tipping point'



CASE EXAMPLE 1: VIRTUAL PALATE

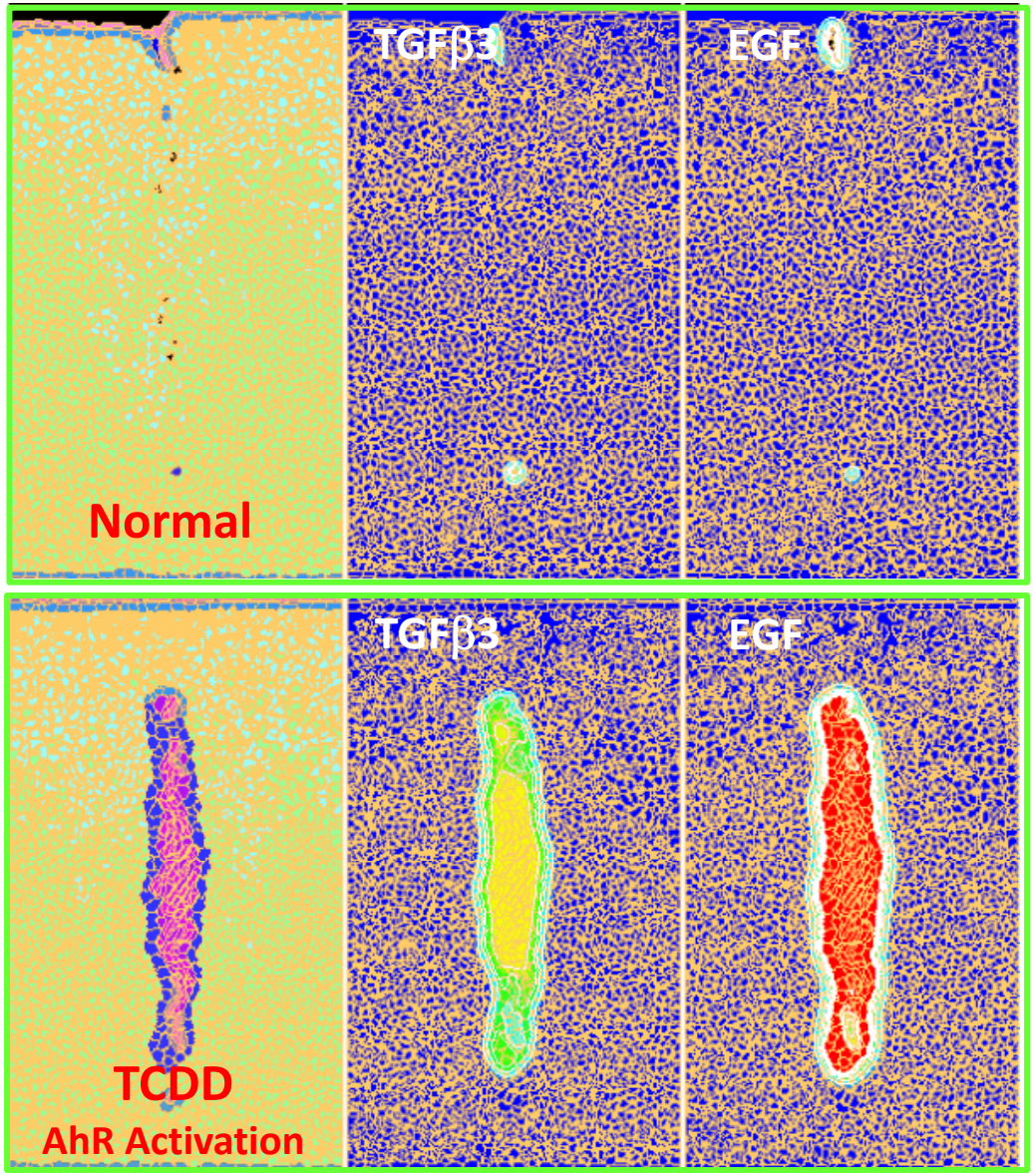


SOURCE: Hutson et al. (manuscript in preparation)

TCDD: FLIPPING THE EGF/TGFβ3 SWITCH

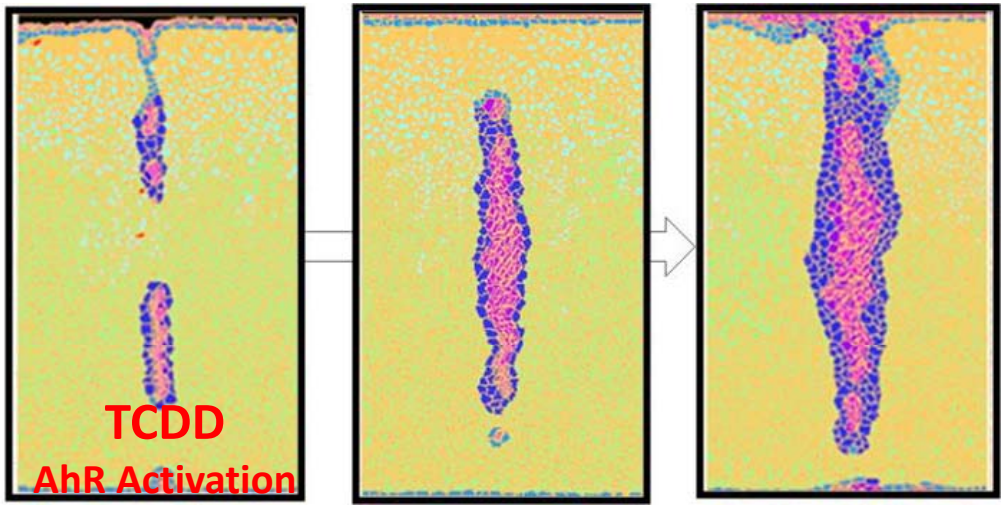
KEY EVENT:
seam breakdown

TCDD
↓
AhR
↓
↑EGF, ↓TGFb

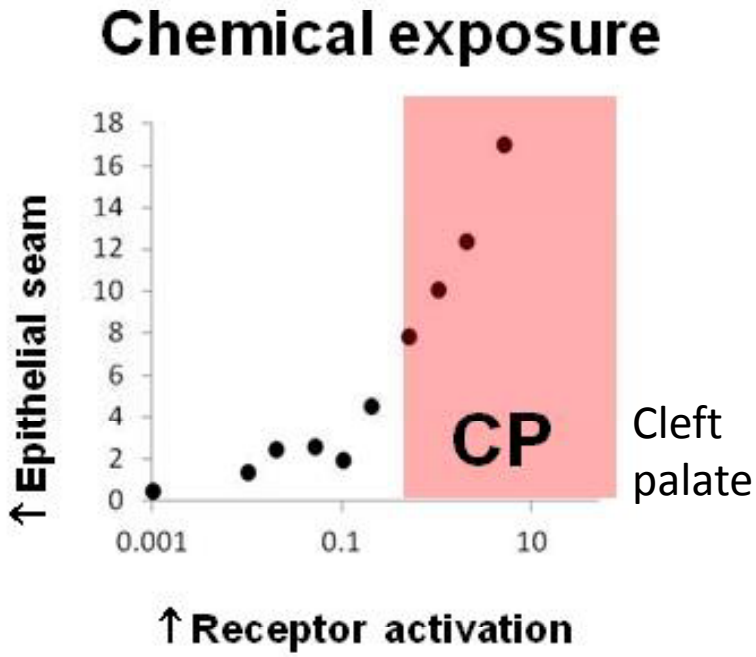


SOURCE: Hutson et al. (manuscript in preparation)

TCDD: ESTIMATING TIPPING POINT



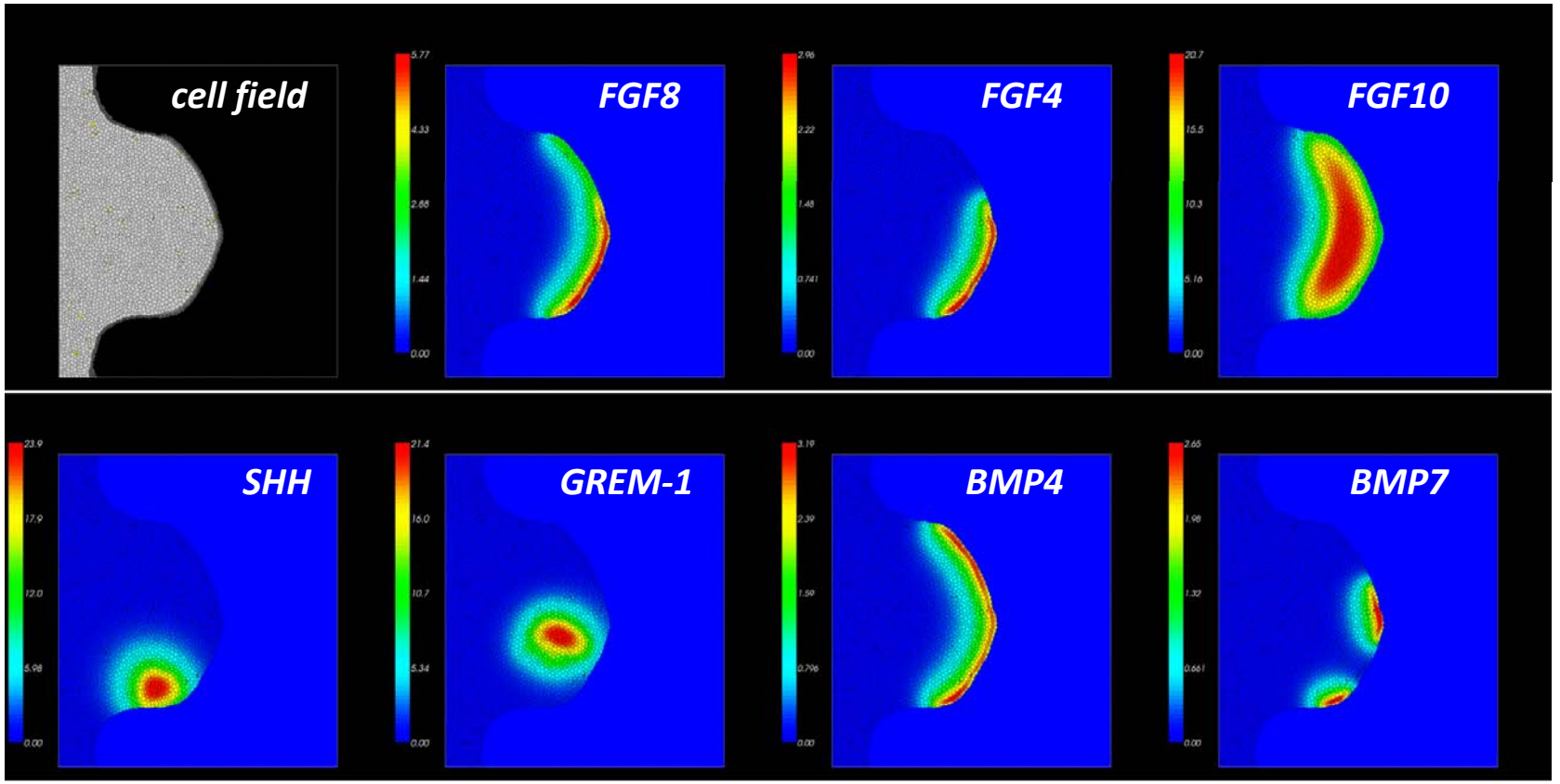
INCREASING DOSE



Cleft palate

SOURCE: Hutson et al. (manuscript in preparation)

CASE EXAMPLE 2: LIMB DEVELOPMENT



SOURCE: Knudsen et al. (manuscript in preparation)

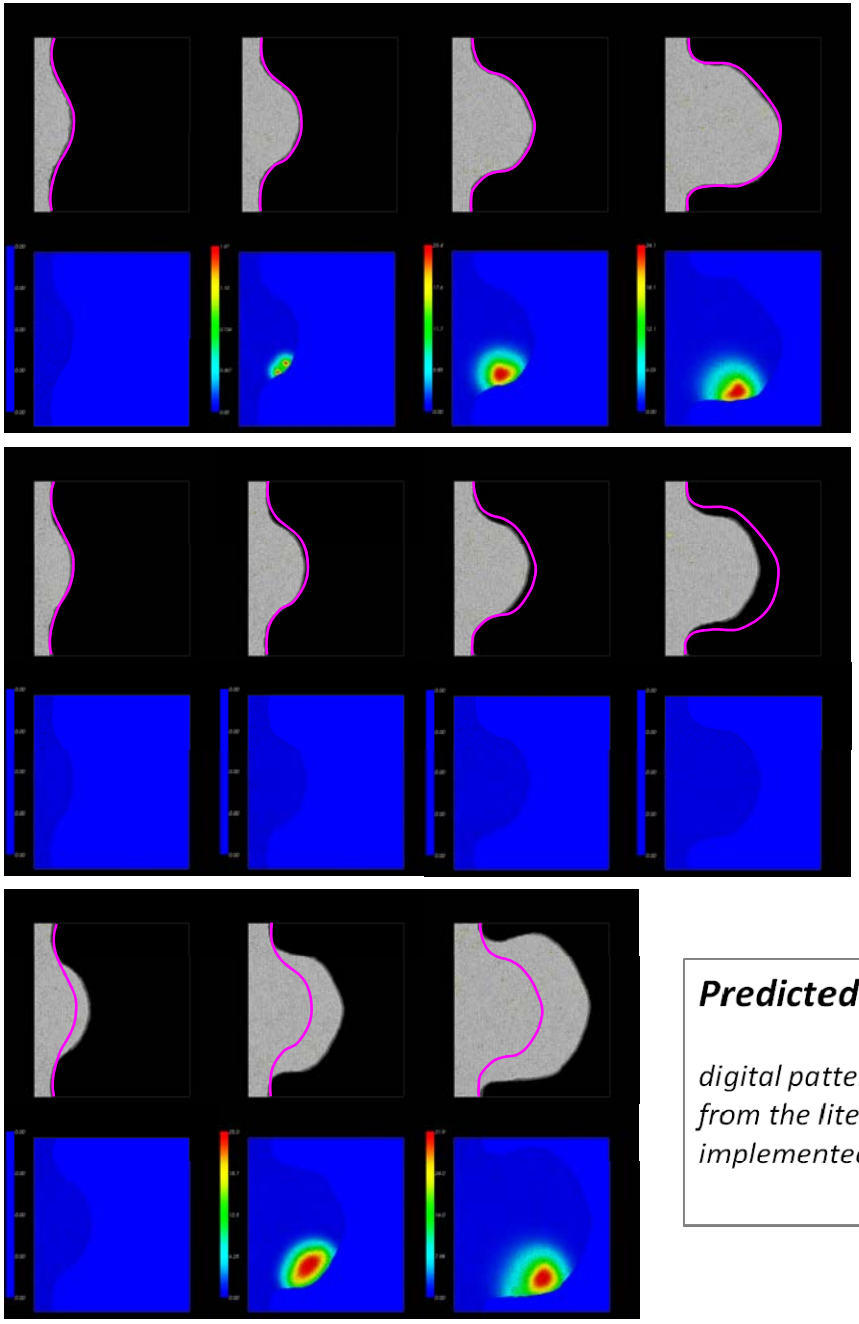
SHH DISRUPTION

Simulated outgrowth

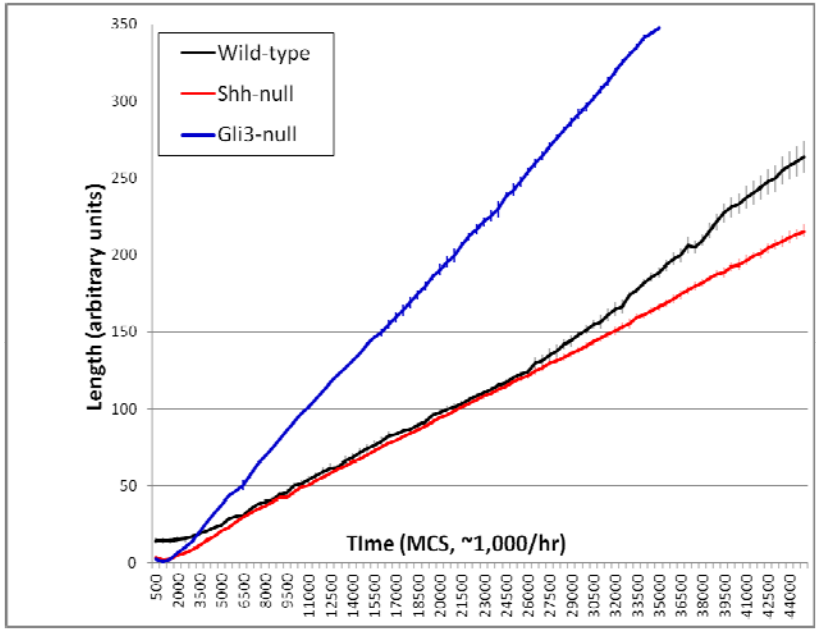
Wild-type

Shh-null

Gli3-null



Rate of elongation *(n=5)*



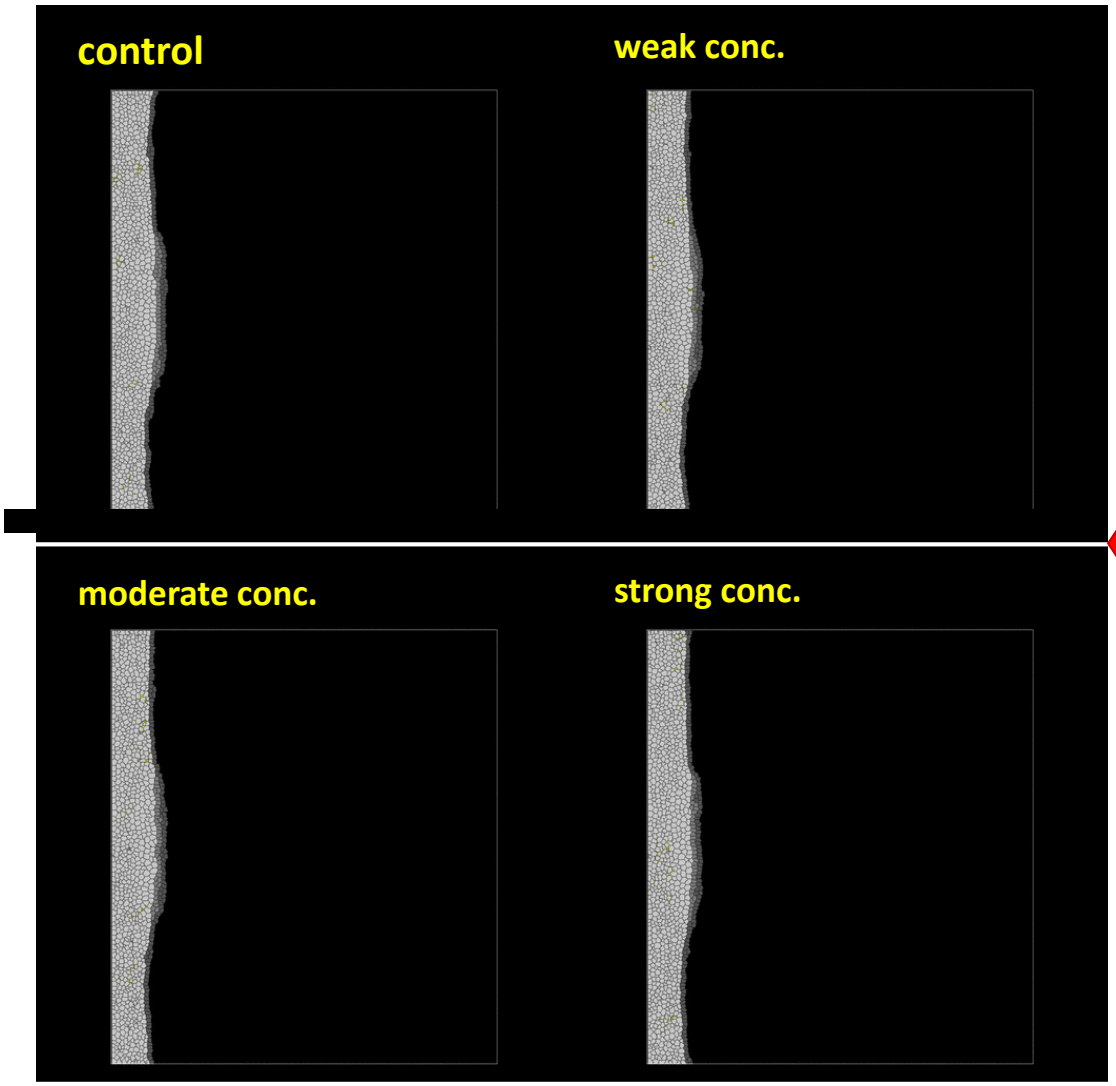
Predicted outcomes

digital patterns inferred from the literature; not yet implemented in the model

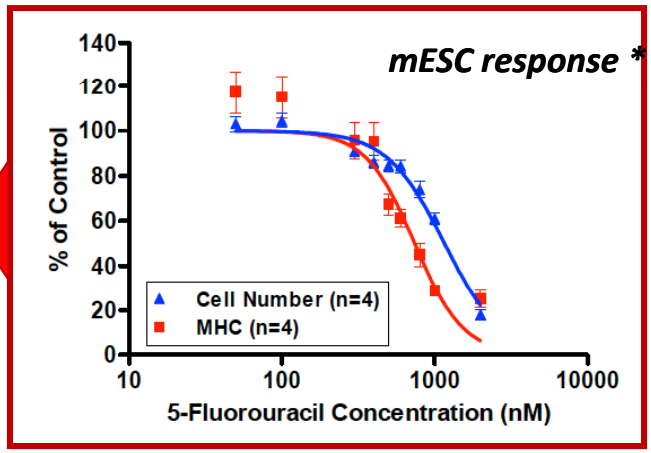
Wild-type *Shh-null* *Gli3-null*

23

'What-If?' - DISRUPTION OF CELL GROWTH – 5FU

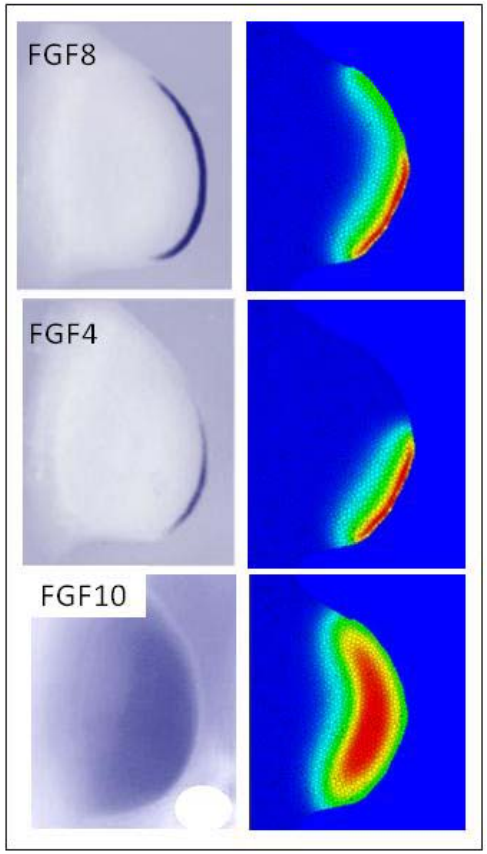


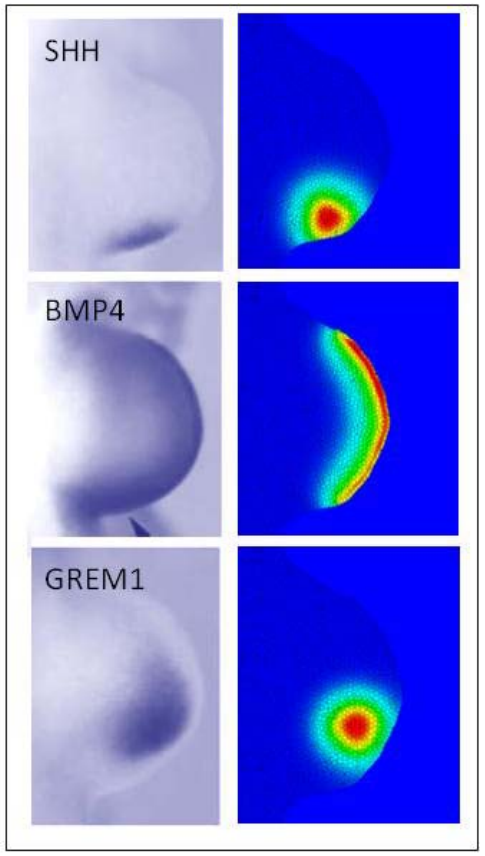
Can read HTS data into the model …



… and predict impact on embryo.

Biological Verification



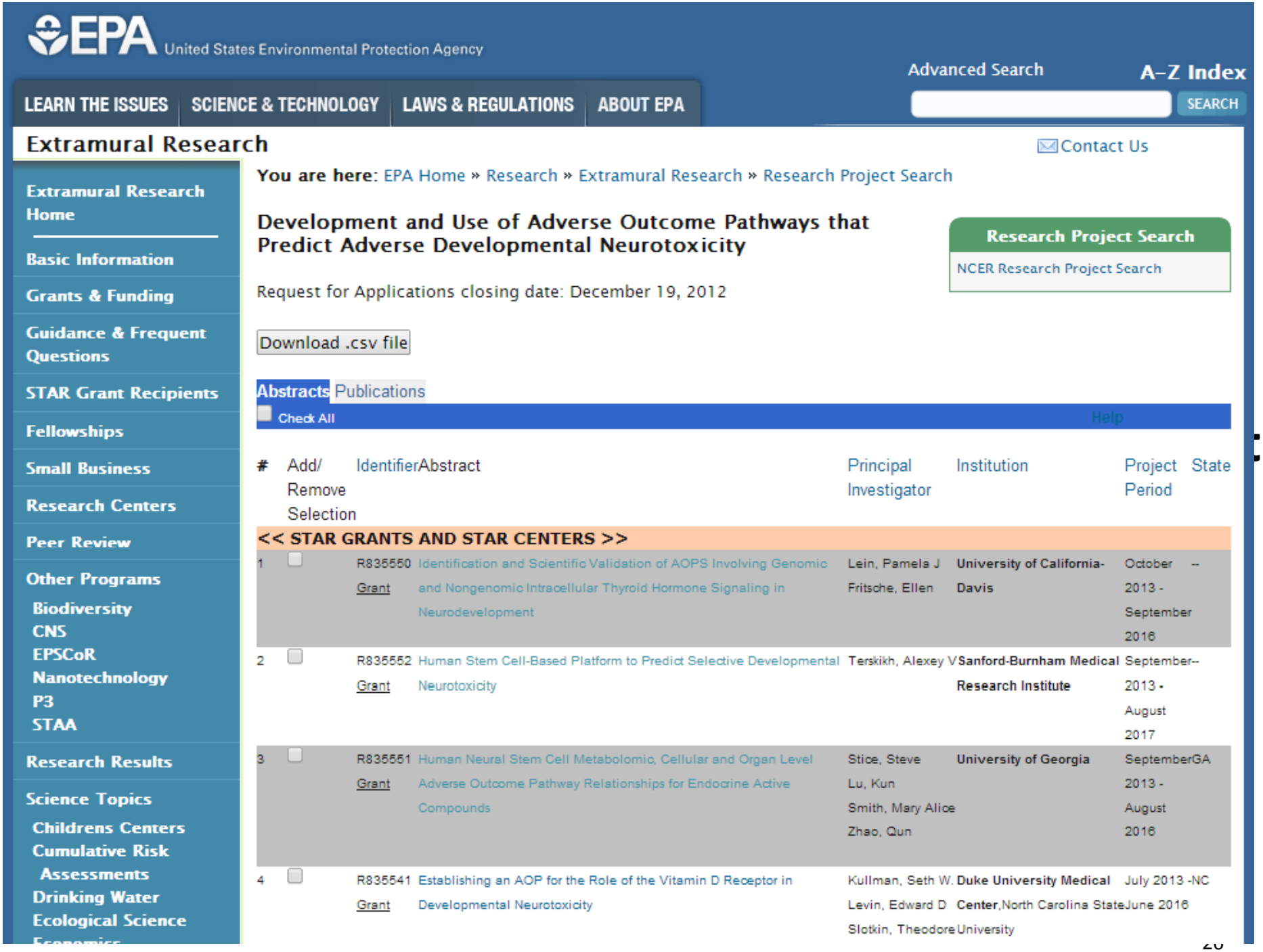


In situ hybridization (mouse literature) vs ABM

- Normal development
- Predicted outcomes

EPA STAR (Science to Achieve Results) Program: currently closed

Organotypic Culture Models for Predictive Toxicology Center



EPA United States Environmental Protection Agency

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Development and Use of Adverse Outcome Pathways that Predict Adverse Developmental Neurotoxicity

Request for Applications closing date: December 19, 2012

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NCER Research Project Search

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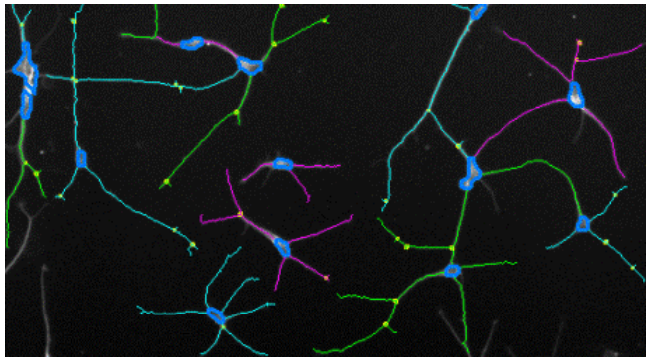
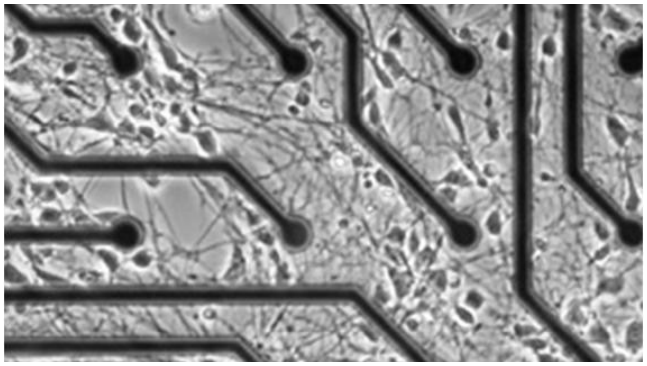
#	Add/ Remove Selection	Identifier	Abstract	Principal Investigator	Institution	Project Period	State
<< STAR GRANTS AND STAR CENTERS >>							
1		R835550 Grant	Identification and Scientific Validation of AOPS Involving Genomic and Nongenomic Intracellular Thyroid Hormone Signaling in Neurodevelopment	Lein, Pamela J Fritsche, Ellen	University of California-Davis	October 2013 - September 2016	--
2		R835552 Grant	Human Stem Cell-Based Platform to Predict Selective Developmental Neurotoxicity	Terskikh, Alexey V	Sanford-Burnham Medical Research Institute	September 2013 - August 2017	--
3		R835551 Grant	Human Neural Stem Cell Metabolomic, Cellular and Organ Level Adverse Outcome Pathway Relationships for Endocrine Active Compounds	Stice, Steve Lu, Kun Smith, Mary Alice Zhao, Qun	University of Georgia	September 2013 - August 2016	GA
4		R835541 Grant	Establishing an AOP for the Role of the Vitamin D Receptor in Developmental Neurotoxicity	Kullman, Seth W. Levin, Edward D Slotkin, Theodore	Duke University Medical Center,North Carolina State University	July 2013 -June 2016	NC

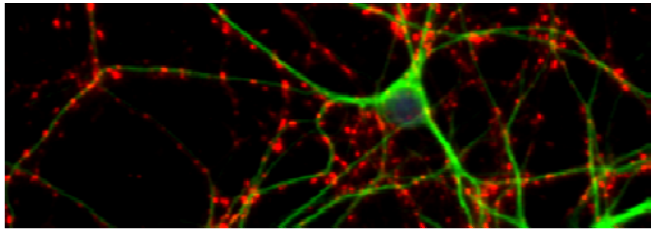
EPA relevant DNT Assays



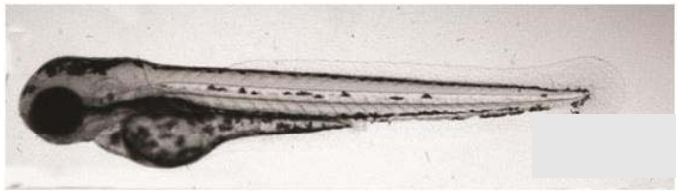
Neurite Outgrowth (B. Mundy)



Network activity (T. Shafer)

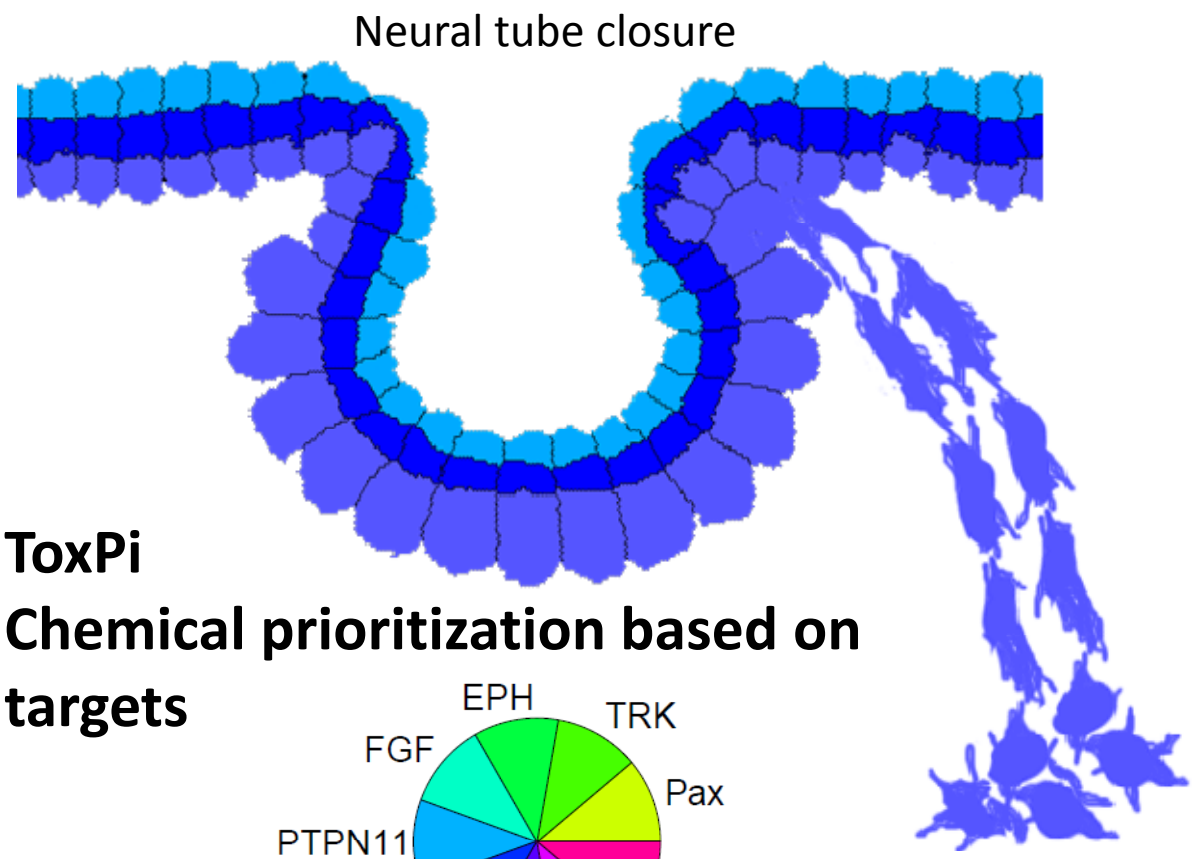


Synaptogenesis (B. Mundy)



Zebrafish (S. Padilla)

Cranial Neural Crest Cell Migration



ToxPi
Chemical prioritization based on targets

Possible ToxCast targets of disruption

-with chemical ranking out of 309

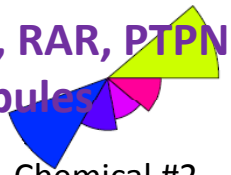
Ecto-mesenchymal transition

- **BMP, Microtubules**

Chemical #10

Migration

- **Ephrins, RAR, PTPN11, PAX, Microtubules**



Chemical #2

Homing, growth, & survival

- **TGFb, FGF, TRK, Microtubules**

Chemical #1

Chemical #5

Moving Science Forward

- **Executable Biology** (13 April 2012): **modeling** how pathways and networks influence tissue patterning and homeostasis **through the actions of individual cells.**

- **Morphogenesis** (7 June 2013): biologically-driven assembly **building human tissues** and mini-organ microsystems **from scratch** – *what the embryo knows best!*

- **Mechanical Forces** (12 October 2012): biophysical design principles in human-on-a-chip microsystems make **complex culture models** more physiologically relevant.

Some Benefits and Challenges For Predictive Toxicology:

- high-throughput hypothesis testing (mechanistic understanding)
- parameter sweeps to isolate key elements (sensitivity analysis)
- pinpointing nascent events underlying 'emergent' biology
- surrogate for missing data or information (knowledge gaps)
- predicting impacts of cellular changes on system dynamics
- probing pathway interactions (convergence, cumulative)
- simulating different exposure scenarios (ADME)
- not a living entity (can only code rules as we understand them)
- finding sweet-spot to enable, but not over-specify performance
- how complex do these systems models need to be (reality check)

VIRTUAL EMBRYO TEAM www.epa.gov/ncct/v-Embryo/

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R Thomas (NCCT), E Cohen-Hubal (dNPD)
K Crofton, J Tietge, J Kenneke (MIs)
T Knudsen and S Hunter (Task Leads)

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M Firestone (EPA - OCHP)
S Darney (EPA – CEH roadmap)

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S Clendenon, A Shirinifard (TIVS – Indiana U)
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C Lau, B Abbott, C Wolf, M Narotsky (EPA-NHEERL)
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S Hutson (Vanderbilt U)