EPA
United States
Environmental Protection
Agency

Predictive Models and Computational Toxicology

Thomas B. Knudsen, PhD
Developmental Systems Biologist
National Center for Computational Toxicology, USEPA

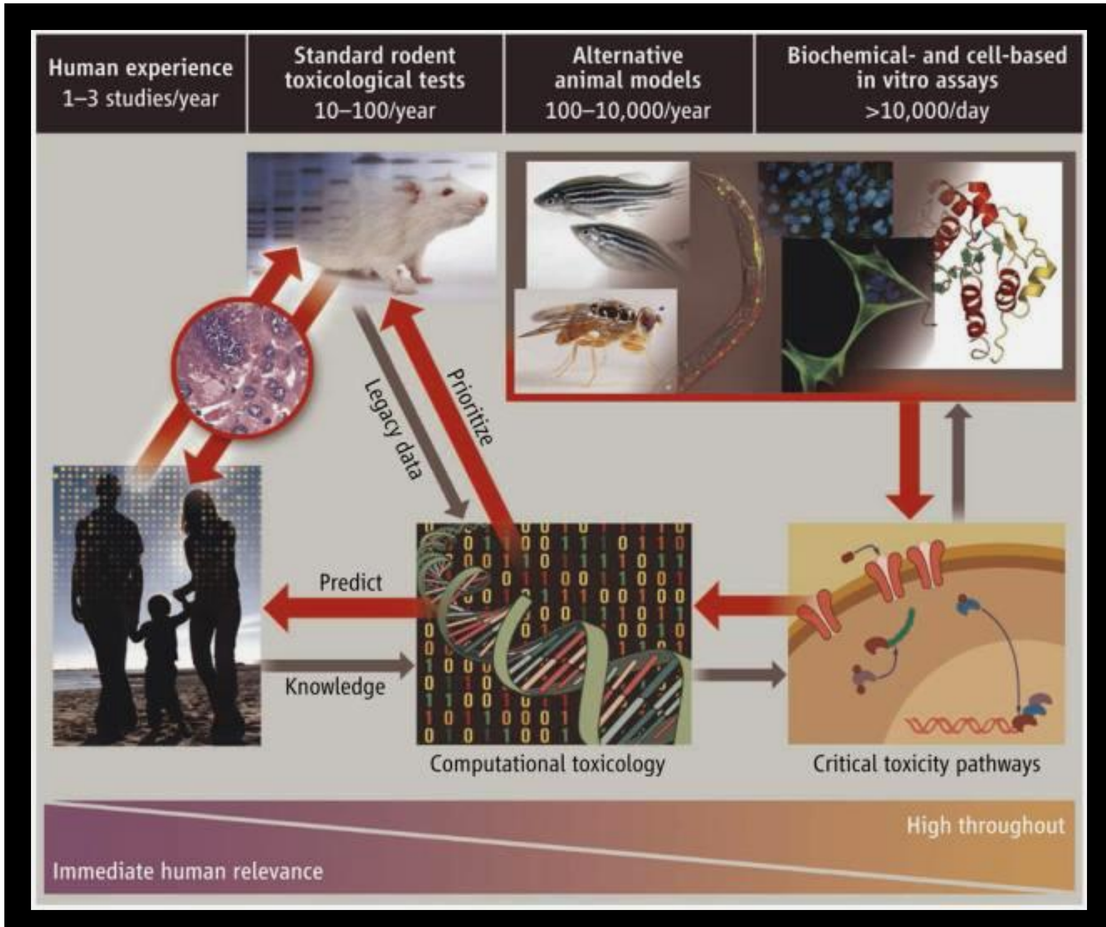
DISCLAIMER: The views expressed in this presentation are those of the author[s] and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

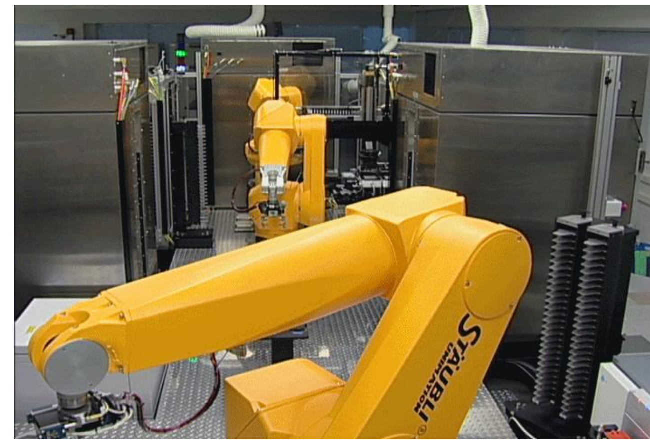
II IBAMTOX, June 16-18, 2013 Ribeirao Preto, Brazil

Problem Statement

- tens of thousands of chemicals, most lack toxicity information especially for reproduction and development
- standard paradigm for toxicity testing is low throughput: animal-based studies focus on 'apical endpoints'
- emerging 21st paradigm: focus on pathways tested *in vitro* using assays amenable to high-throughput screening (HTS)
- computational challenge: to integrate vast arrays of *in vitro* data into *in silico* models predictive of *in vivo* toxicity

Tox21 Vision:
Transforming Toxicity Testing





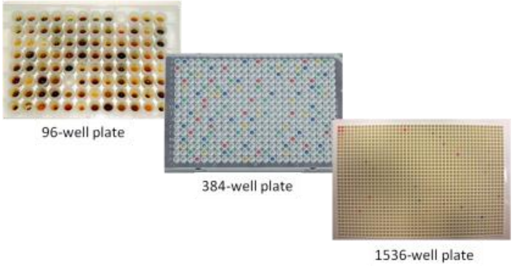
National Center for Advancing Translational Sciences (NCATS)
http://www.ncats.nih.gov/

SOURCE: Collins, Gray and Bucher (2008) Toxicology. Transforming environmental health protection. Science 319: 906

Computational Toxicology:
high-throughput screening (HTS)

- **ToxCast:** EPA research effort profiling >1060 chemicals across >700 *in vitro* assays (27M data points). **http://www.epa.gov/ncct/toxcast/**
  - **Phase-I:** 310 data-rich chemicals (primarily pesticides) having over 30 years of traditional animal studies valued at $2B (completed 2011).
  - **Phase-II:** adds 767 chemicals (eg, industrial and consumer products, food additives, failed drugs) extend the broader chemical landscape (Sept 2013).
  - **Phase-III:** adds 1001 compounds in a subset of assays (2014).
- **Tox21:** partnership of federal agencies.
  - brings chemical inventory to 10,000
  - >50 HTS *in vitro* assays (ongoing)

ToxCast Assays



Assay Provider
ACEA
Apredica
Attagene
BioReliance
BioSeek
CeeTox
CellzDirect
Tox21/NCATS
NHEERL MESC
NHEERL Zebrafish
NovaScreen (Perkin Elmer)
Odyssey Thera
Vala Sciences

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

Assay Design
viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter

Readout Type
single
multiplexed
multiparametric

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

Species
human
rat
mouse
zebrafish
sheep
boar
rabbit
cattle
guinea pig

Tissue Source

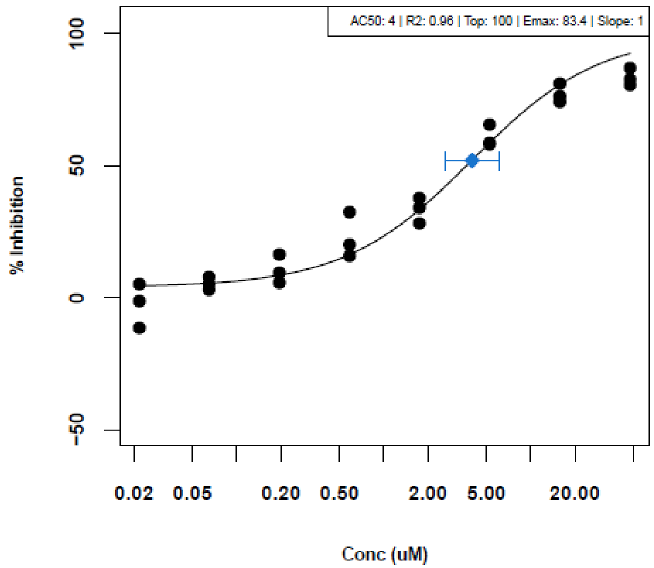
Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

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

List of assays and related information at: **http://www.epa.gov/ncct/**

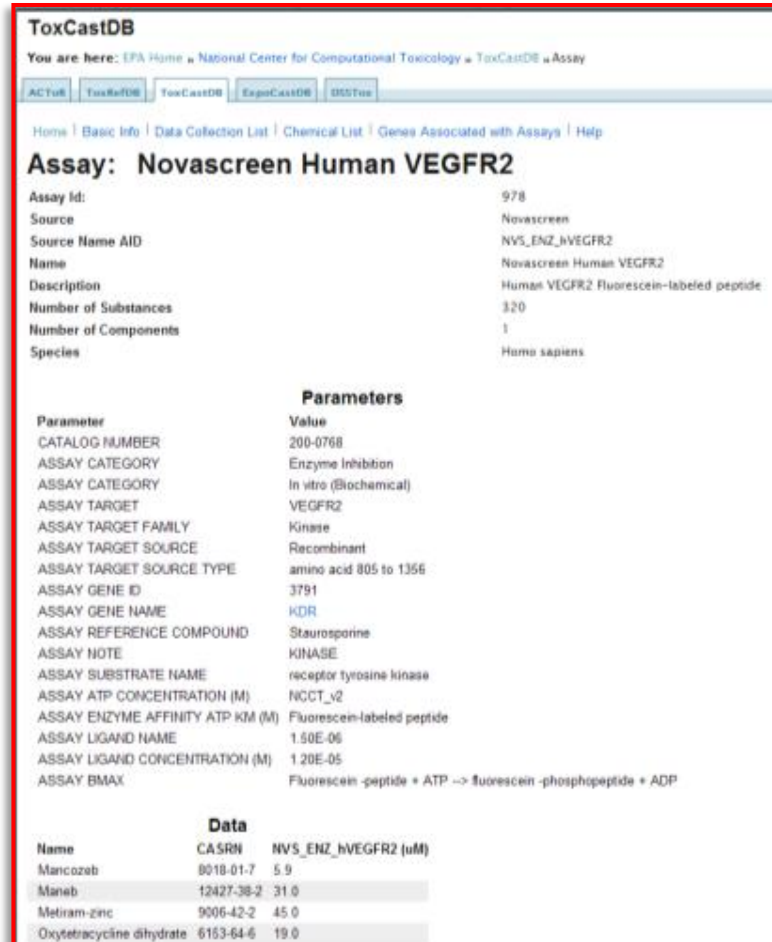
ToxCastDB

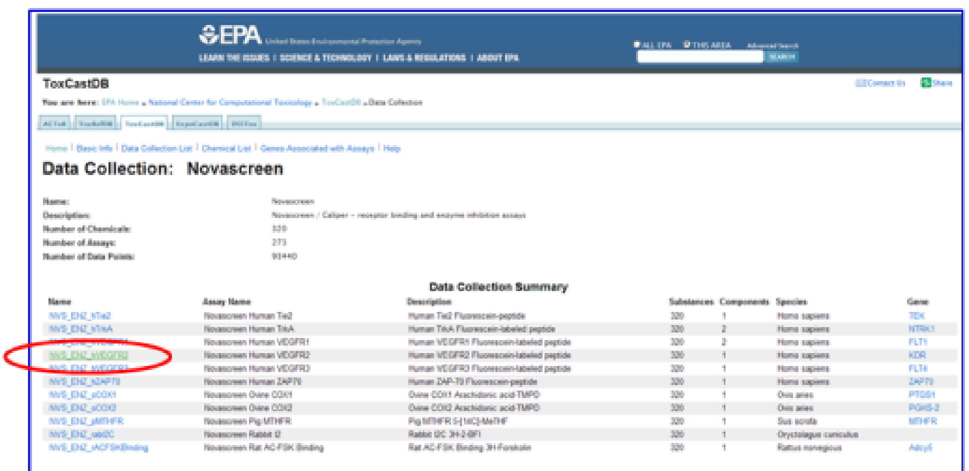
http://actor.epa.gov/actor/faces/ToxCastDB/DataCollection.jsp



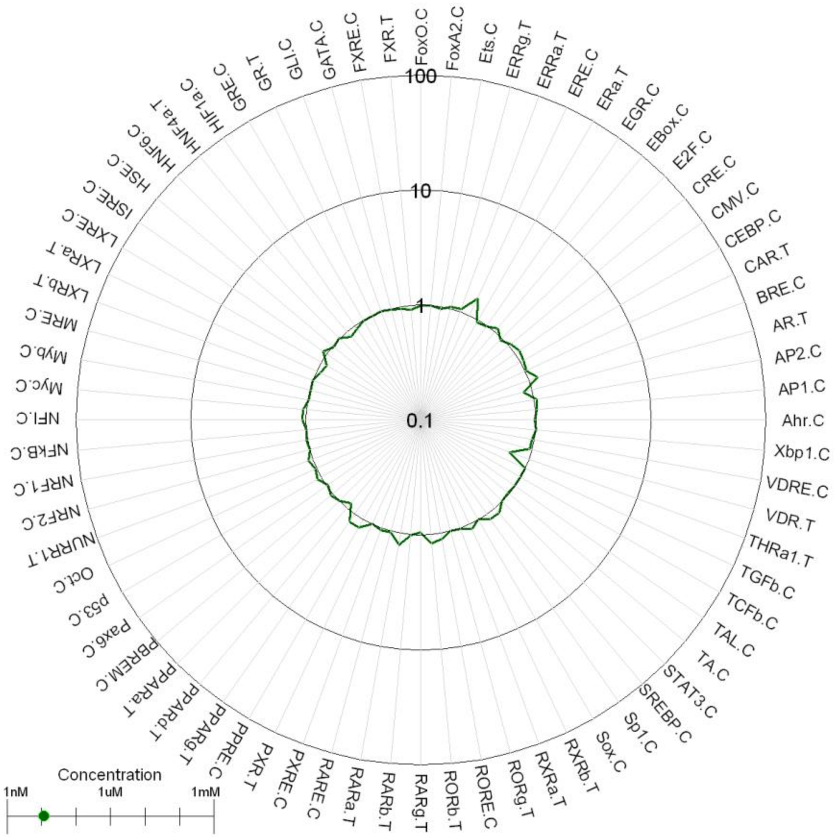
AC50 concentration producing a 50% change

LEC lowest effect concentration





EXAMPLE: reporter gene activation (Attagene)



Spider plot maps the activity of 72 reporter gene pathways tested in ToxCast

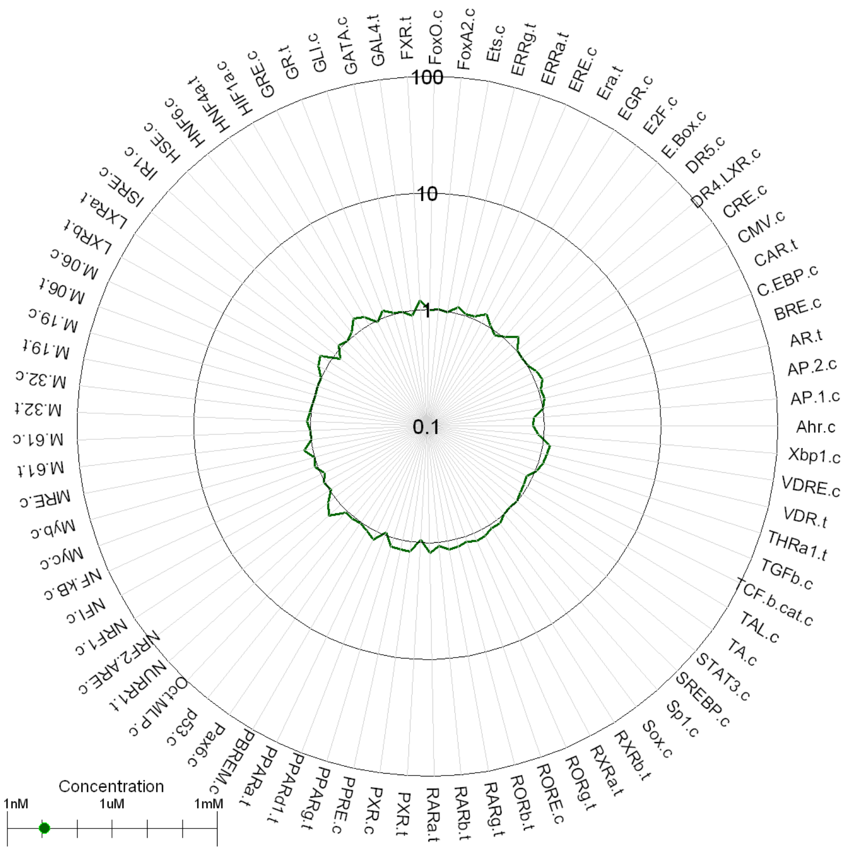
Animation shows concentration response

Example: a pharmaceutical tested in ToxCast Phase II

Sunburst: loss of specificity

SOURCE: M Martin, NCCT

EXAMPLE: reporter gene activation (Attagene)



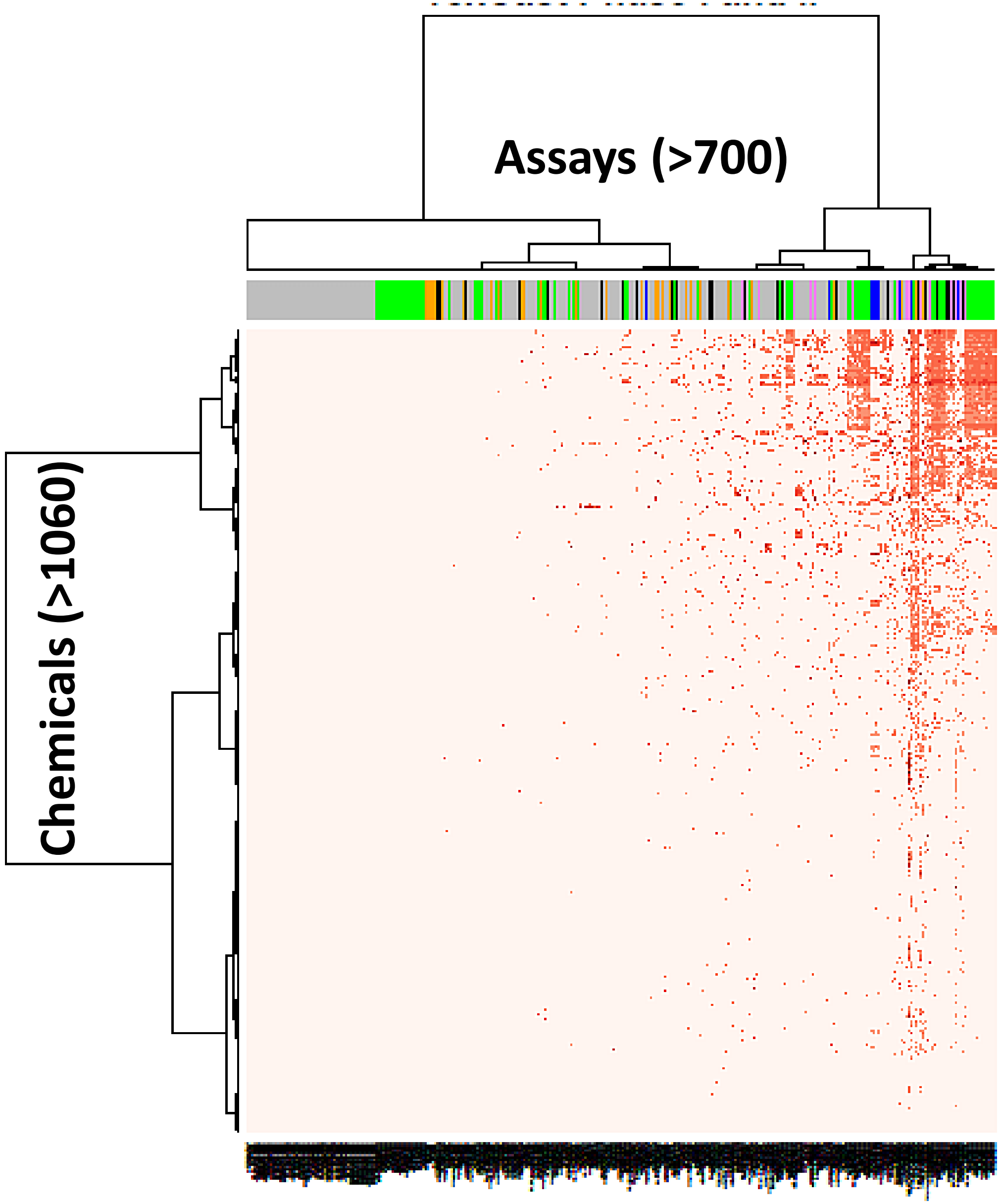
Spider plot maps the activity of 72 reporter gene pathways tested in ToxCast

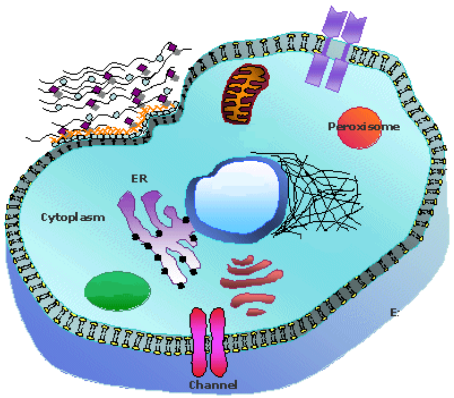
Example: concentration response for an environmental chemical (Pentachlorophenol)

Sunburst: approaching cytotoxicity

SOURCE: M Martin, NCCT

In vitro profiling: we can now begin to group chemicals by their 'bioactivity profiles' and look for signatures that predict toxicity.





Mining the Predictive Signatures:

What assays best correlated with selected endpoints?

Endpoints
(*in vivo*)

ToxRefDB

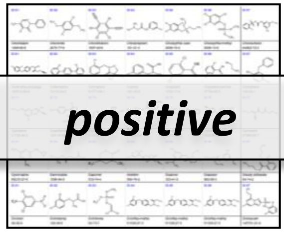
Endpoint Class	Number of Chemicals		
	Rat	Rabbit	Overlap
Developmental (dLEL)	146	106	65
Fetal Weight Reduction (FWR)	87	45	20
Malformation (MAL)*	127	75	39
Skeletal (axial)	113	53	25
Skeletal (appendicular)	49	22	7
Skeletal (cranial)	40	19	3
Urogenital (renal)	[illegible]	2	0
Urogenital (ureteric)	11	2	0
Jaw/Hyoid	[illegible]	6	0
Cleft Lip/Palate	10	2	0
Neurosensory (eye)	2	4	0
Neurosensory (brain)	7	5	0
Body Wall (somatic)	5	1	0
Viscera (splanchnic)	4	8	0
Cardiovascular (heart)	2	3	0
Cardiovascular (major vessels)	1	3	0
Prenatal Loss (PNL)	86	136	47

LEL

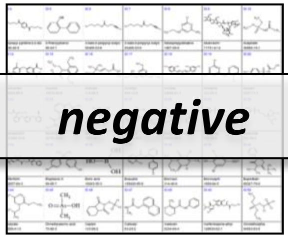
no LEL

Chemicals
(*ToxCast*)

309 compounds



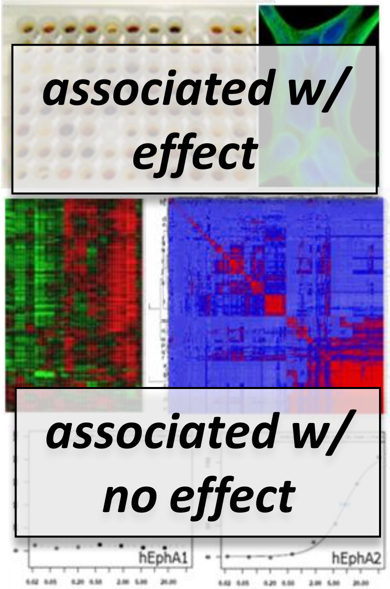
positive



negative

Effects
(*in vitro*)

ToxCastDB



associated w/ effect

associated w/ no effect

ToxCast predictive signatures (1^{st} generation)

❖ **machine-learning models anchored to apical endpoints**

liver tumors: Judson et al. 2010, Env Hlth Persp 118: 485-492
hepatocarcinogenesis: Shah et al. 2011, PLoS One 6(2): e14584
developmental tox: Kleinstreuer et al. 2011, Tox App Pharm 257(1):111-21
rat-rabbit prenatal devtox: Sipes et al. 2011, Tox Sci 124: 109-127
rat fertility: Martin et al. 2011, Biol Reprod 85: 327-339
zebrafish development: Sipes et al. 2011, Birth Defects Res C 93: 256-267
cancer hallmarks: Kleinstreuer et al. 2012, Tox Sci, doi:10.1093/toxsci/kfs285

❖ **prediction models anchored to pathways or processes**

endocrine disruption: Reif et al. 2010, Env Hlth Persp 118: 1714-1720
microdosimetry: Wambaugh and Shah 2010, PLoS Comp Biol 6: e1000756
mESC differentiation: Chandler et al. 2011, PLoS One 6(6): e18540
HTP risk assessment: Judson et al. 2011, Chem Res Toxicol 24: 451-462
angiogenesis: Kleinstreuer et al. 2011, Env Hlth Persp 119: 1596-1603
vascular AOP: Knudsen and Kleinstreuer, 2011, Birth Def Res C 93:312-323
multi-scale simulation: Kleinstreuer et al., 2013, PLoS Comp Biol (in press)

Predictive signature for developmental toxicity

Feature	Description	Weight
RAR	Retinoic Acid receptor	0.58
GPCR	G-Protein-Coupled Receptors	0.55
TGFβ	Transforming Growth Factor β	0.38
MT	Microtubule organization	0.30
SENS_CYP	Cytochrome P450 (sensitive)	0.26
AP1	Activator protein 1	0.24
SLCO1B1	Organic anion transporter 1B1	0.11
CYP	CYPs (other)	0.06
HLA-DR	MHC complex	-0.38
PXR	Pregnane X receptor	-0.24
IL8	Interleukin 8	-0.23
PGE2	Prostaglandin E2 response	-0.18

*Multivariate **Rat** Model*
71% balanced accuracy

Feature	Description	Weight
CCL2	Chemokine ligand 2 (MCP1)	1.15
IL	Interleukin (1a and 8)	0.39
CYP	Cytochrome P450	0.24
TGFβ	Transforming Growth Factor β	0.28
MESC	Mouse ES cells (J1)	0.13
SULT2A1	Sulfotransferase	-0.26
PGE2	Prostaglandin E2 response	-0.15

*Multivariate **Rabbit** Model*
74% balanced accuracy

Phase-I models connected to relevant biological processes in prenatal DevTox

SOURCE: Sipes et al (2011) Toxicol Sci 124

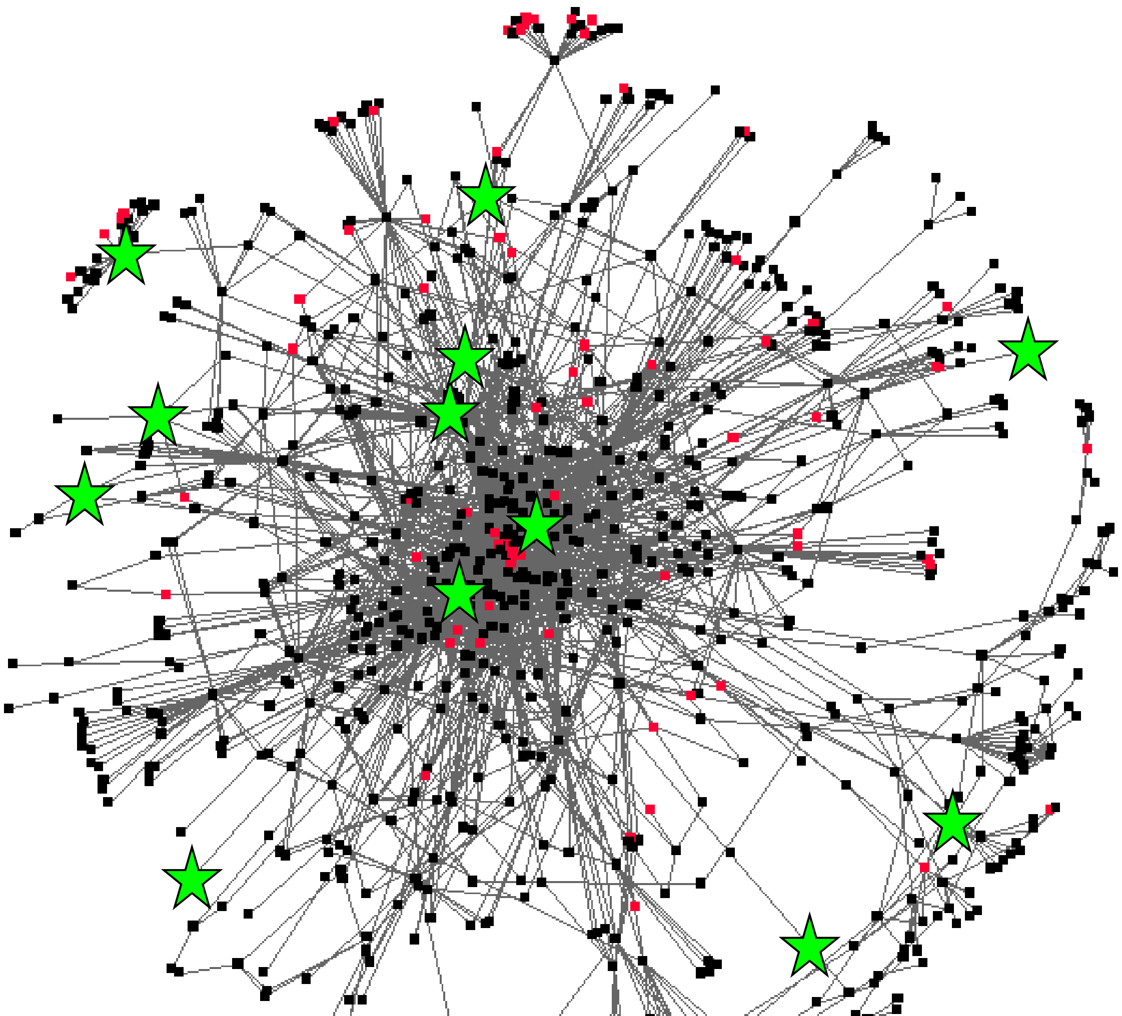
Features mapped by 'biological process'

univariate DevTox features
multivariate DevTox features

processes related to neovascularization (vasculogenesis and/or angiogenesis)

Feature	Description	Weight
RAR	Retinoic Acid receptor	0.58
GPCR	G-Protein-Coupled Receptors	0.55
TGFβ	Transforming Growth Factor β	0.38
MT	Microtubule organization	0.30
SENS_CYP	Cytochrome P450 (sensitive)	0.26
AP1	Activator protein 1	0.24
SLCO1B1	Organic anion transporter 1B1	0.11
CYP	CYPs (other)	0.06
HLA-DR	MHC complex	-0.38
PXR	Pregnane X receptor	-0.24
IL8	Interleukin 8	-0.23
PGE2	Prostaglandin E2 response	-0.18

Feature	Description	Weight
CCL2	Chemokine ligand 2 (MCP1)	1.15
IL	Interleukin (1a and 8)	0.39



HYPOTHESIS: disruption of embryonic blood vessel formation is a direct target for some developmental effects

- Gene Ontology (GO) and Mammalian Phenotype (MP) browsers of MGI database (**http://www.informatics.jax.org/**) for **neovascularization**:
  - abnormal vasculogenesis [MP:0001622; 72 genotypes, 73 annotations]
  - abnormal angiogenesis [MP:0000260; 610 genotypes, 894 annotations]

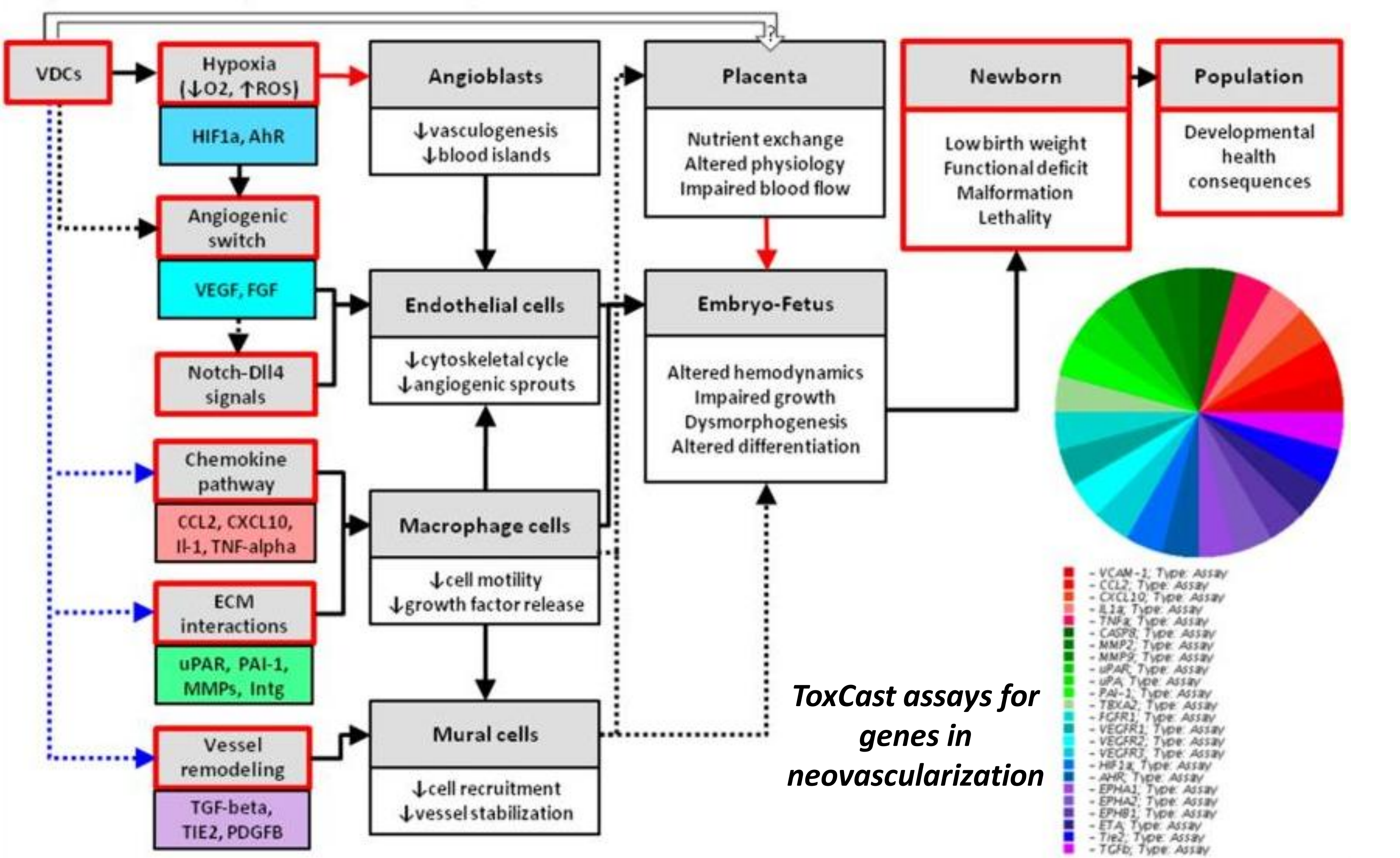
- 65 genes with roles in vasculogenesis or angiogenesis linked to ToxCast assays, 50 had evidence of abnormal embryonic vascular development in MGI

Overlap between ToxCast assay targets and abnormal vascular phenotypes from genetic mouse models.

ToxCast Gene Target *	MP Annotated Term	ToxCast Assays
AHR	patent ductus venosus, abnormal vascular regression	ATG_Ahr_CIS, NCGC_AhR,
BMPR2	decreased angiogenesis	ATG_BRE_CIS
CASP8	abnormal vitelline vasculature morphology	NVS_ENZ_hCASP8
CCL2	decreased angiogenesis, abnormal physiological neovascularization, choroidal neovascularization	BSK_3C_MCP1, BSK_4H_MCP1, BSK_KF3CT_MCP1, BSK_LPS_MCP1, BSK_SAg_MCP1, BSK_SM3C_MCP1
CEBPB*	abnormal vasculogenesis, absent organized vascular network	ATG_C_EBP_CIS, ATG_CRE_CIS

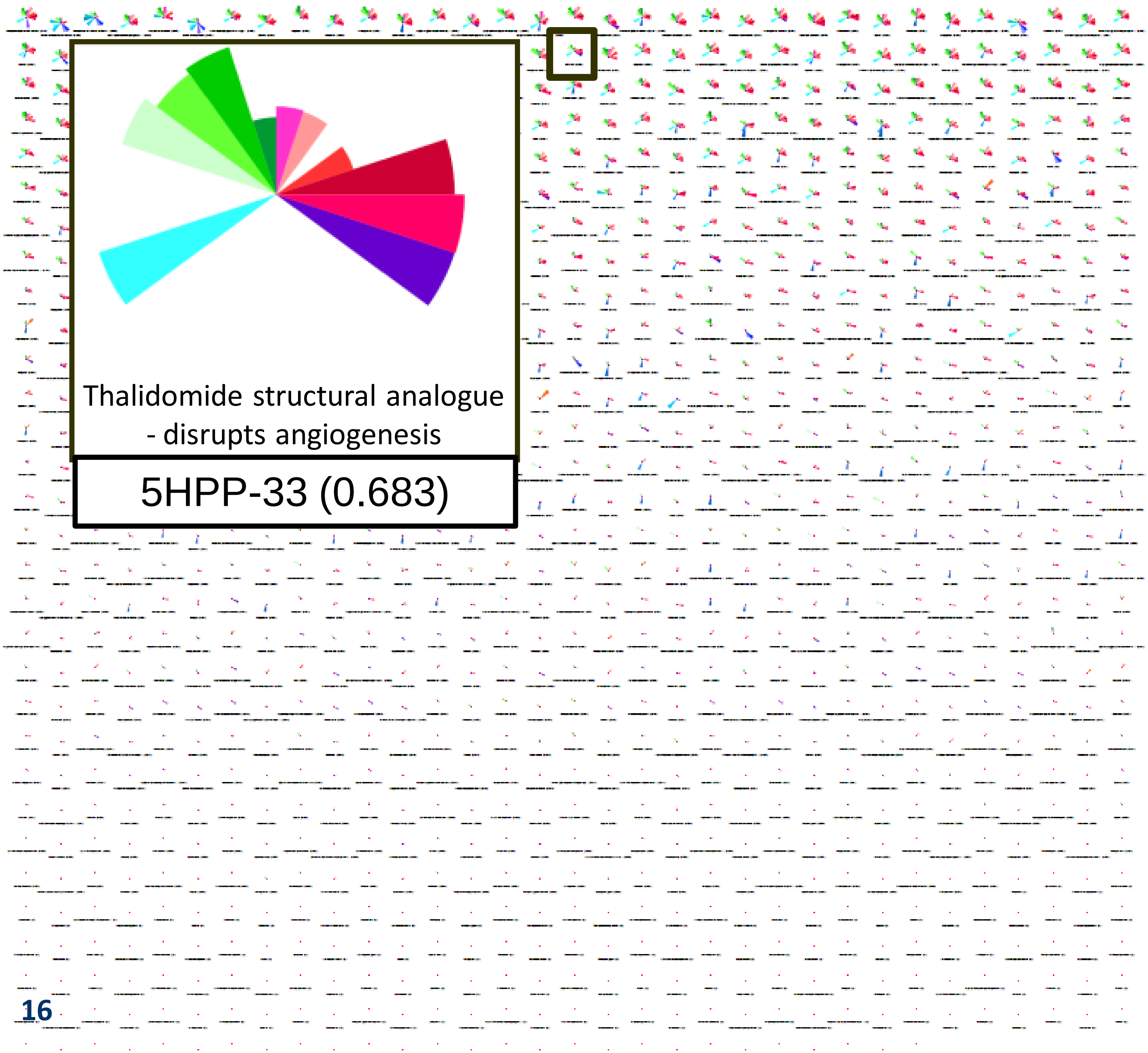
SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res. C

Proposed Adverse Outcome Pathway (AOP) for embryonic vascular disruption: based on what we know

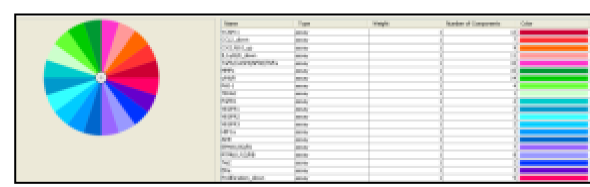


SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res. C

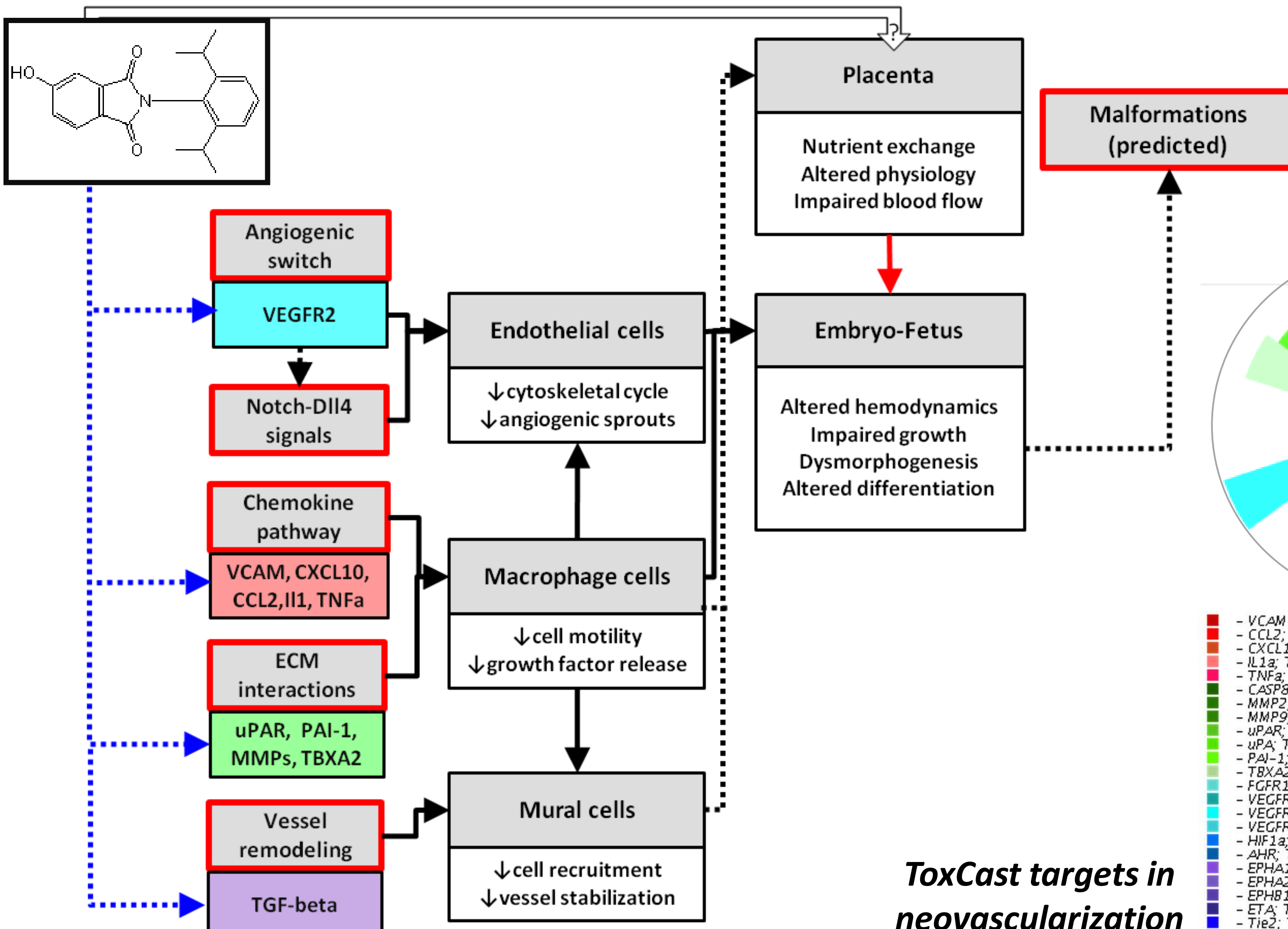
ToxPi ranking by pVDC score: 1060 ToxCast compounds

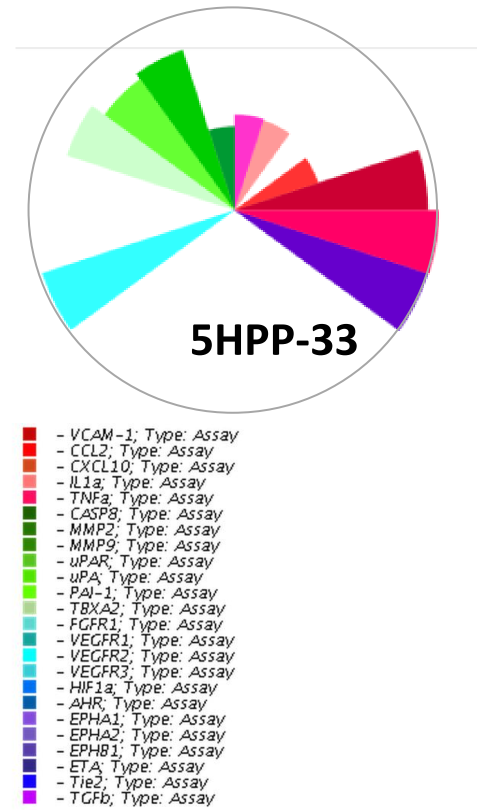


Toxicity Prioritization Index
(ToxPi) for vascular disruption



Proposed AOP for 5HPP-33

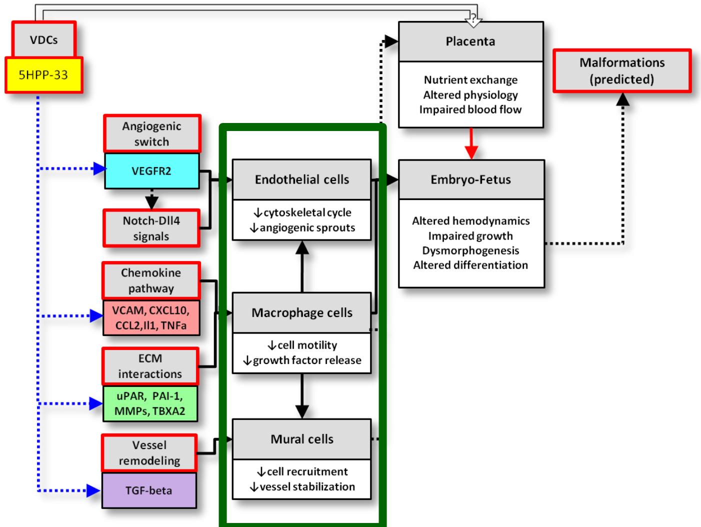


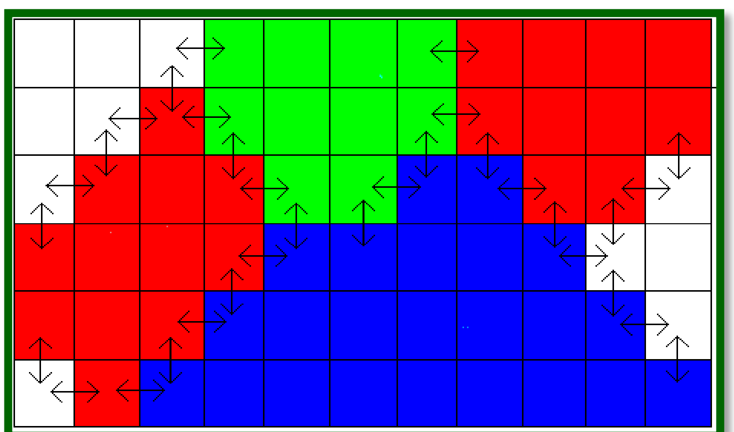


ToxCast targets in neovascularization

SOURCE: Kleinstreuer et al (2013) PLoS Comp Biol (in press)

cellular *Agent-Based Models* (ABMs)





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)

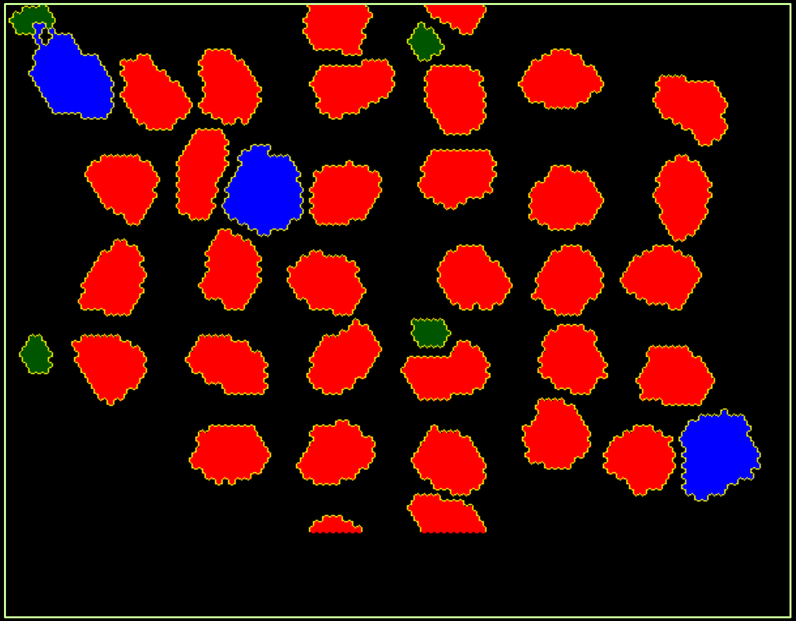
www.CompuCell3D.org

ABMs address cellular systems biology:

- each cell is an ‘agent’ (unit of autonomous decision)
- simulation is driven by biological networks and rules

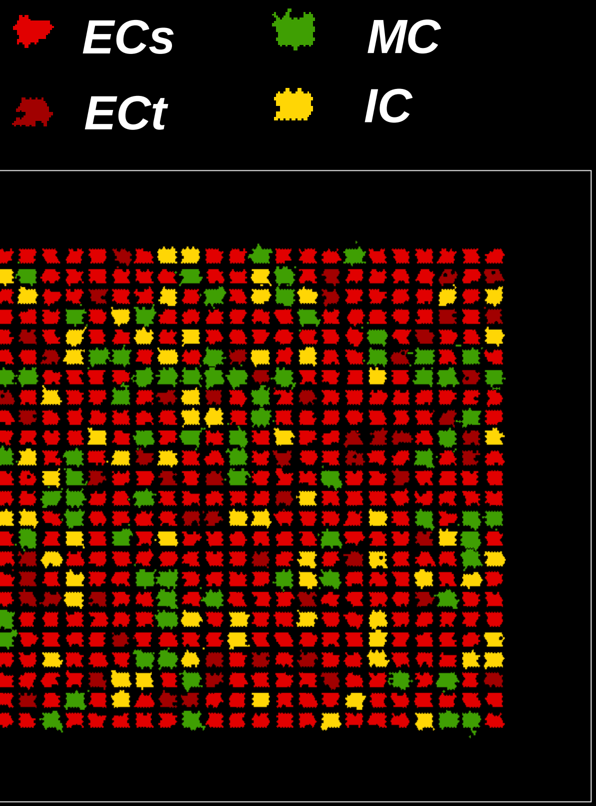
In vitro

In silico



macrophage
RBC
bug

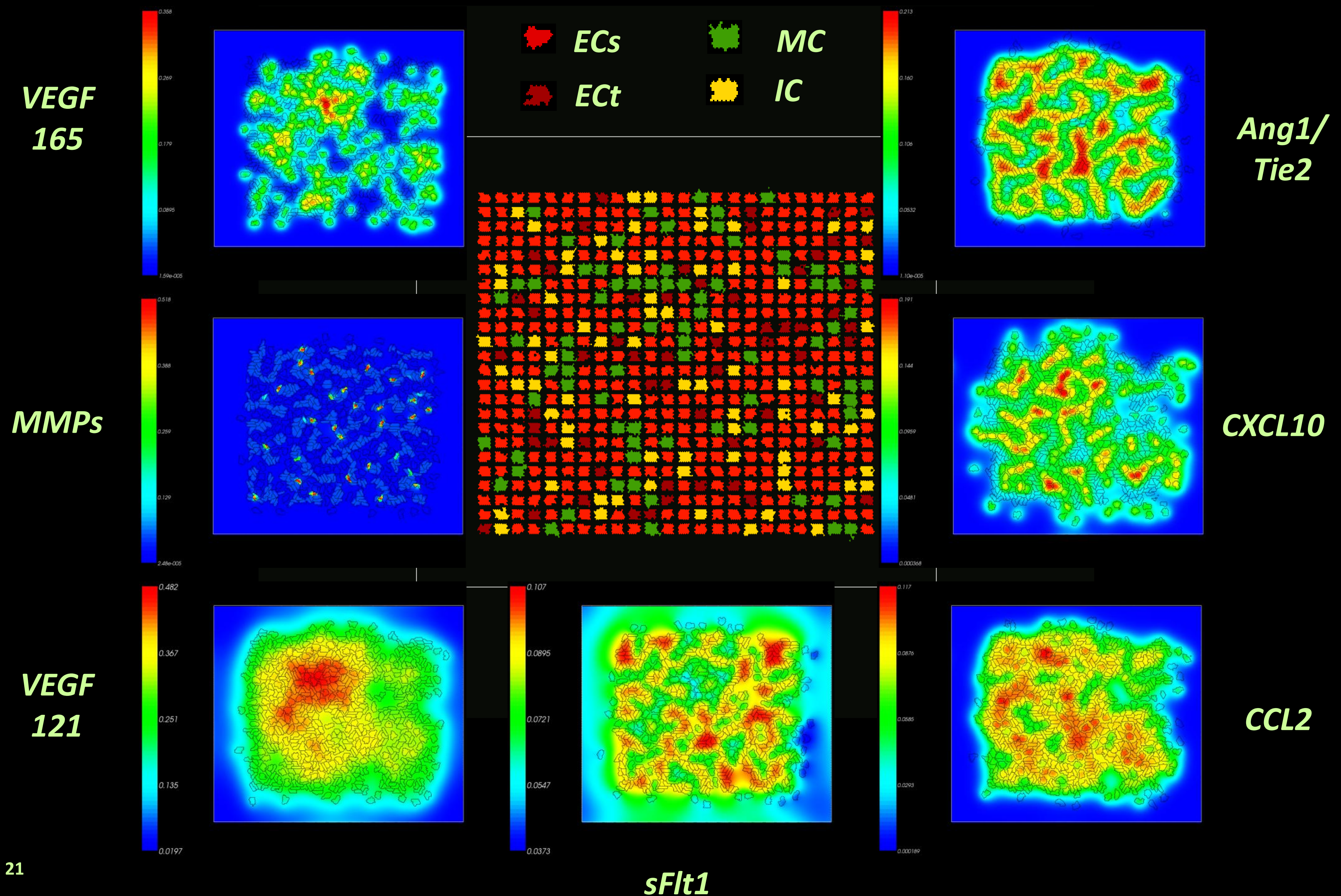
Multicellular ABM of vasculogenesis



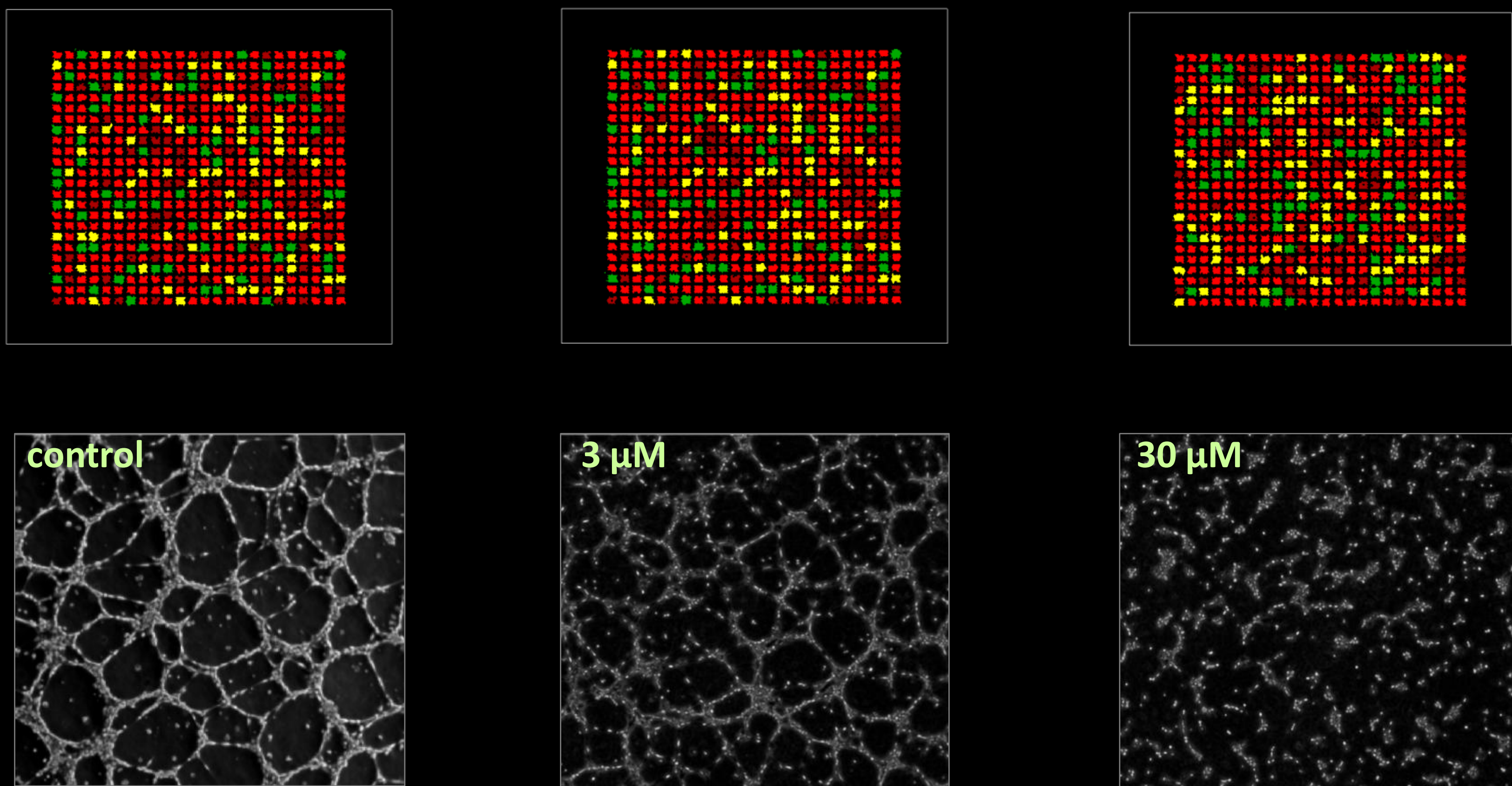
- ABM forms capillary network from endothelial cells (red), inflammatory cells (yellow), and mural cells (green)

SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol (in press)

Multicellular ABM of vasculogenesis



- Cell ABM simulation perturbed with ToxCast HTS data captures the 5HPP-33 effect and concentration-response

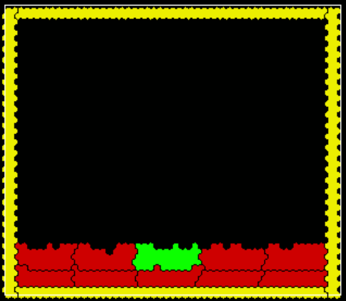


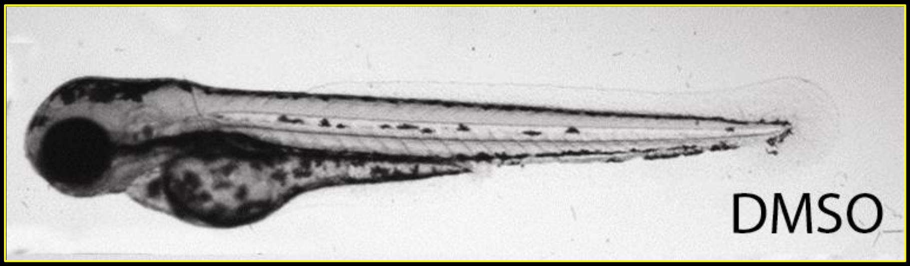
SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol (in press)

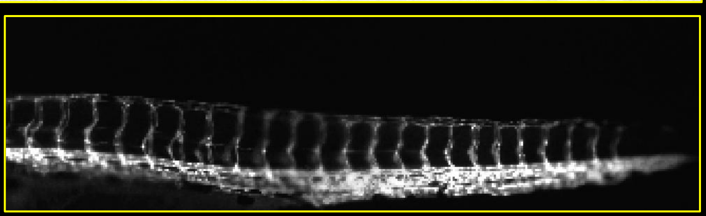
VEGFR2 inhibitor:

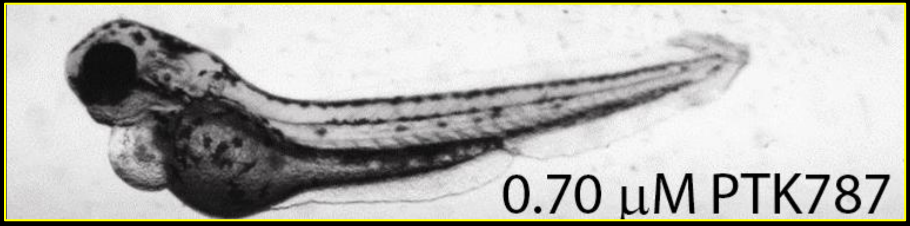
transgenic zebrafish embryos

sprouting

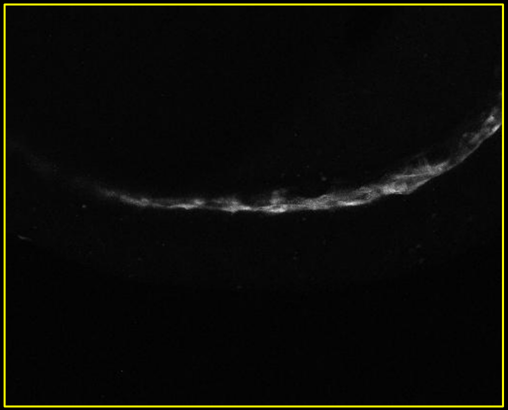




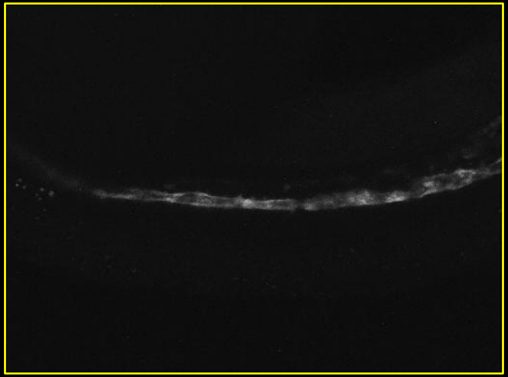




DMSO



0.70 μM
PTK787



T Tal, S Padilla
US EPA

C McCollum, M Bondesson
University of Houston

Case for Thalidomide Embryopathy

CRBN
cereblon (proteasome)

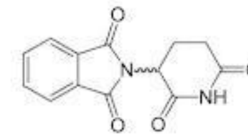
cell-cell signaling
molecular gradients (FGF8)

cellular behaviors
growth and apoptosis

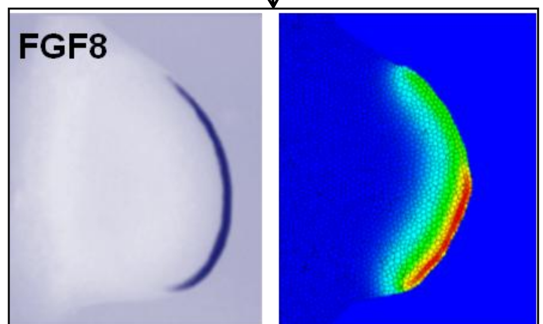
embryonic vasculature
vascular disruption

early limb-buds
dysmorphogenesis

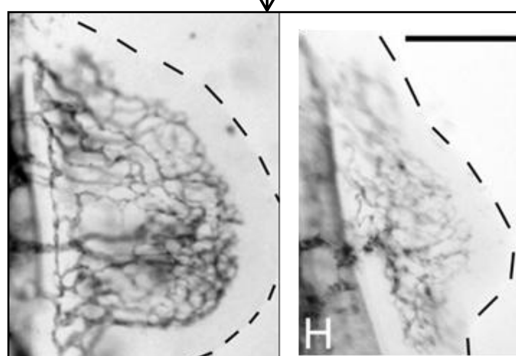
birth defects
limb malformations



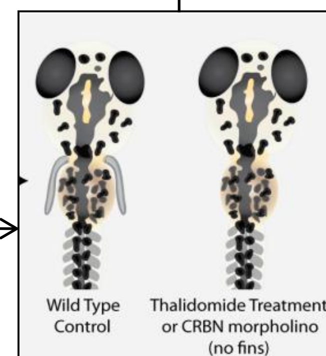
Thalidomide



Short or missing limbs in humans, monkeys, rabbits, zebrafish (but not rodents)



Therapontos et al. 2009, PNAS 106

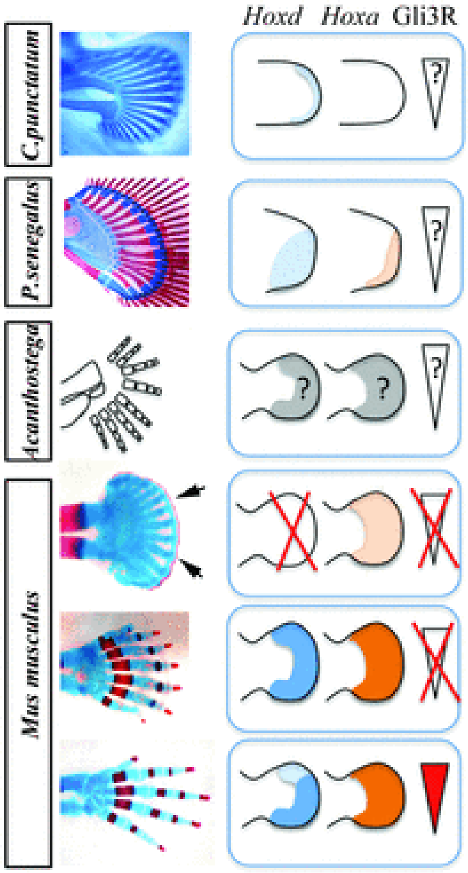


Taylor et al. 2010, Cell Com.Signal.

Digital Morphogenesis

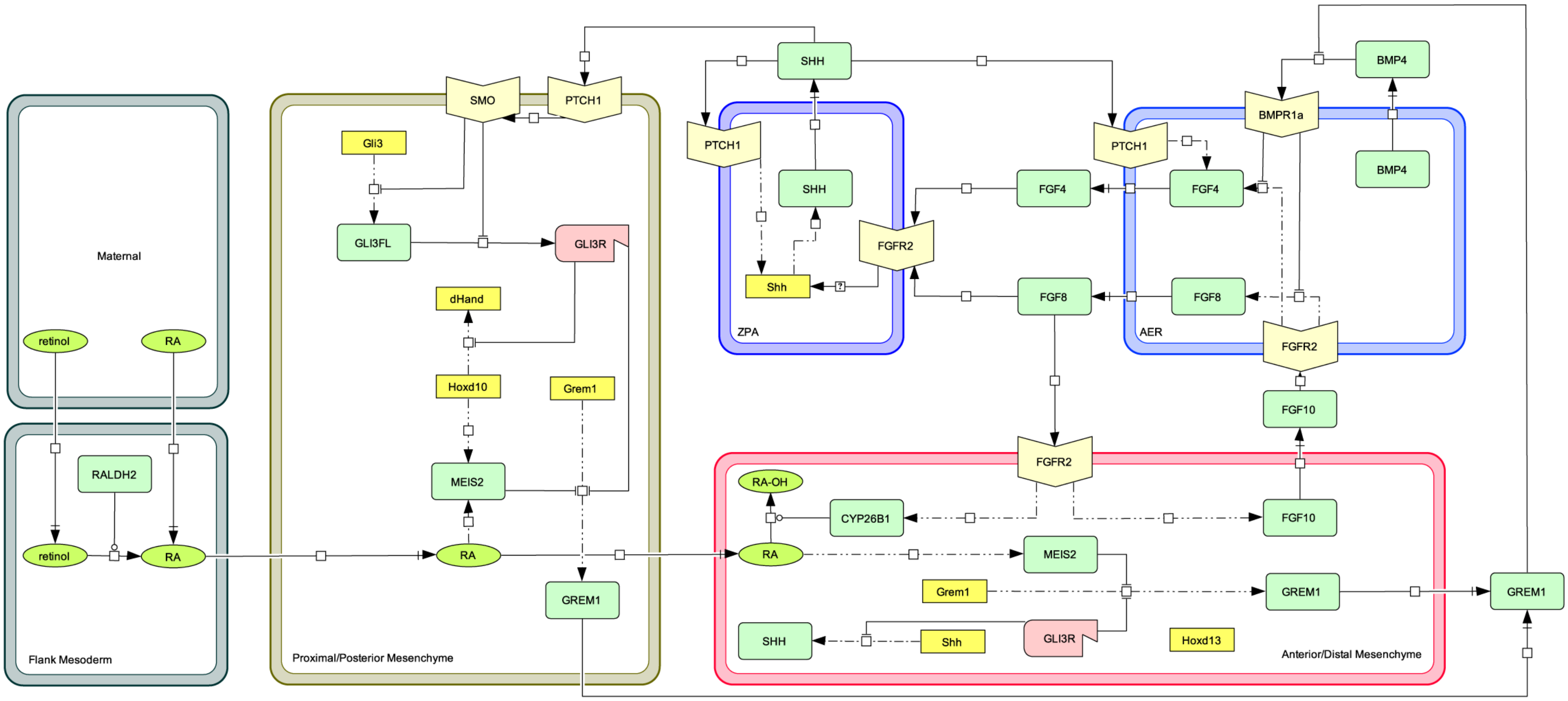
Boot et al. (2008) Nat Met 5: 609

Vogel (2012) Science 338: 1406



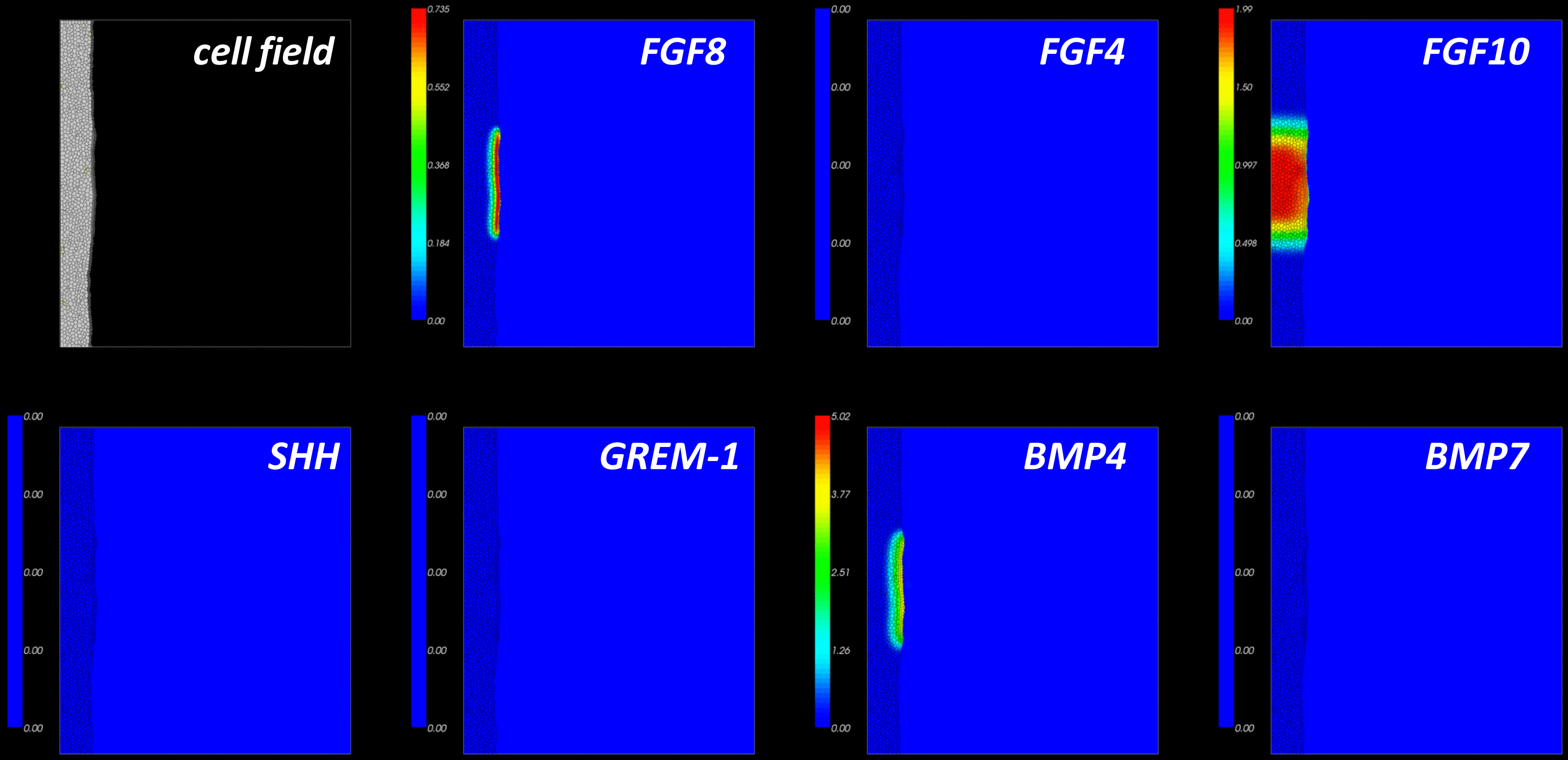
Sheth et al. (2012) Science 338: 1476

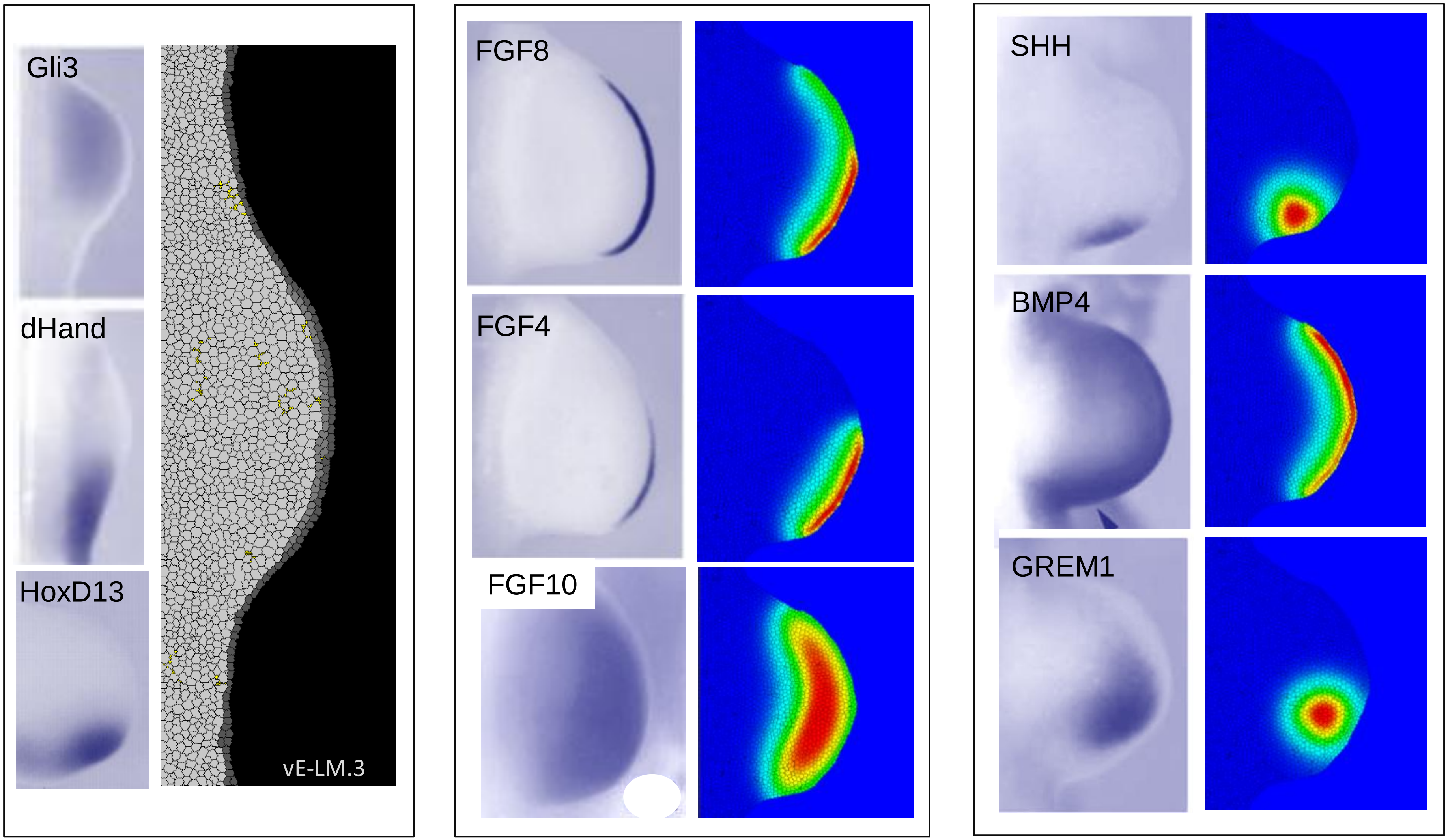
LIMB OUTGROWTH Control Network



SOURCE: Knudsen et al. (in preparation)

- This cell ABM simulates signal propagation during hindlimb-bud outgrowth in early mouse embryos (~42h)





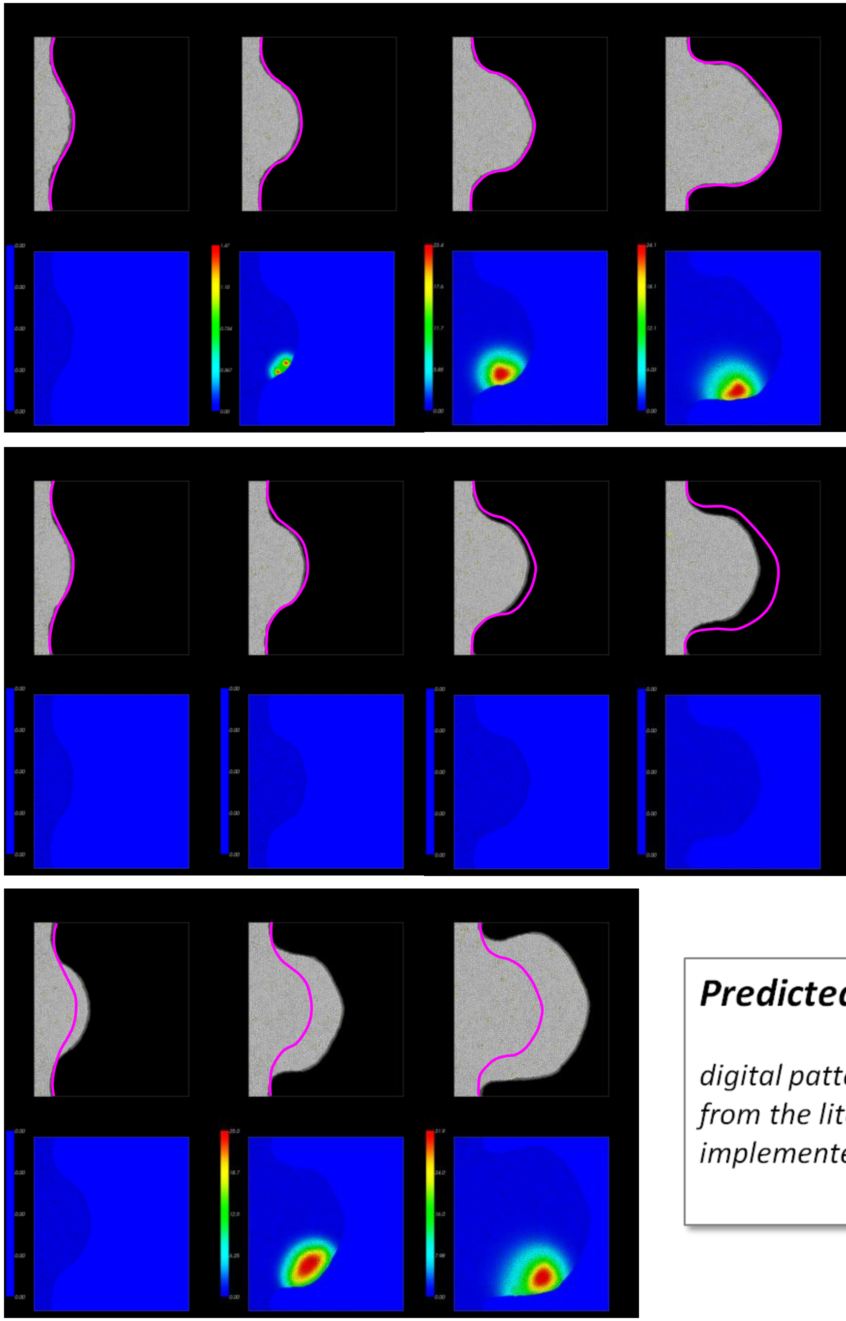
ISH (mouse literature) vs ABM

Simulated outgrowth

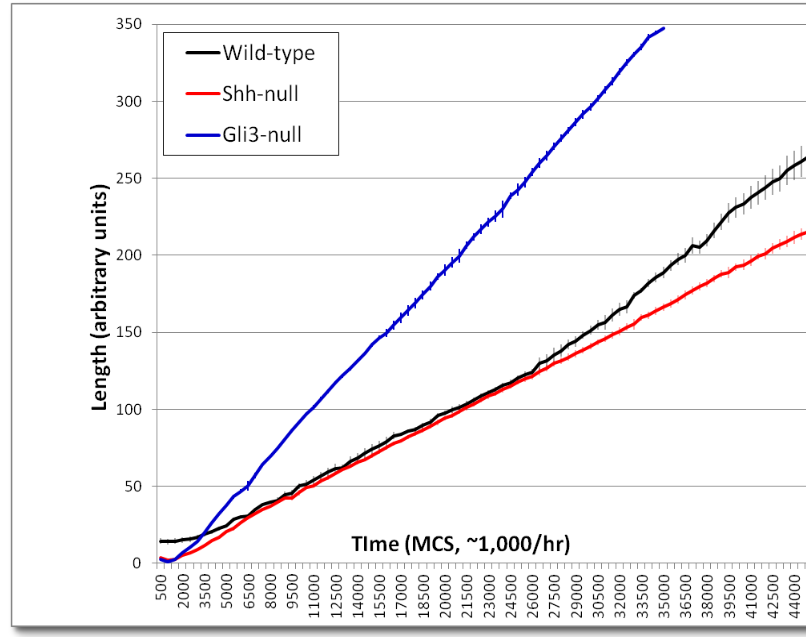
Wild-type

Shh-null

Gli3-null



Rate of elongation *(n=5)*



Predicted outcomes

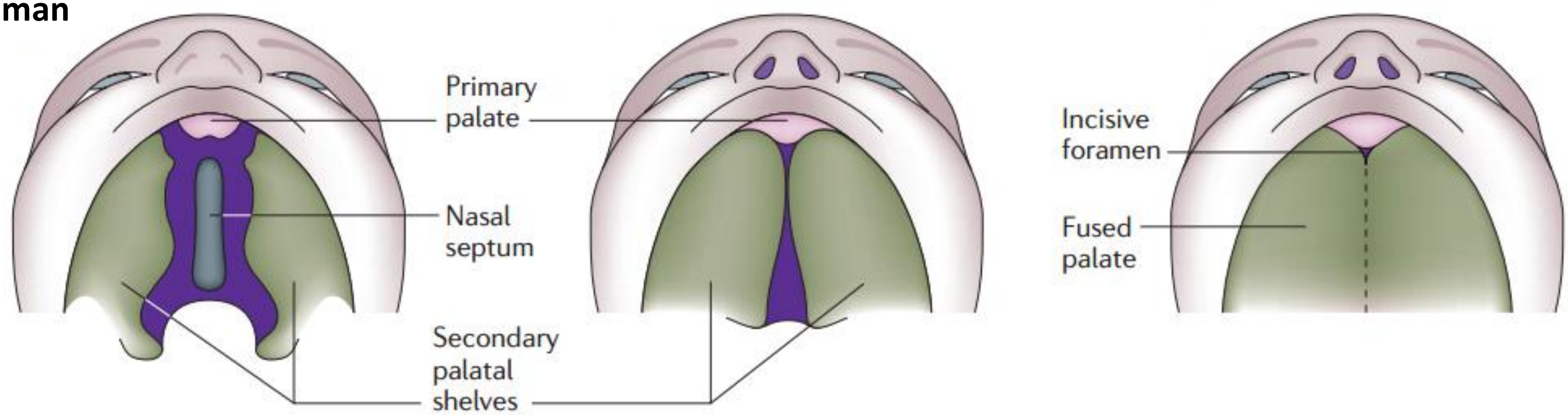
digital patterns inferred from the literature; not yet implemented in the model

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

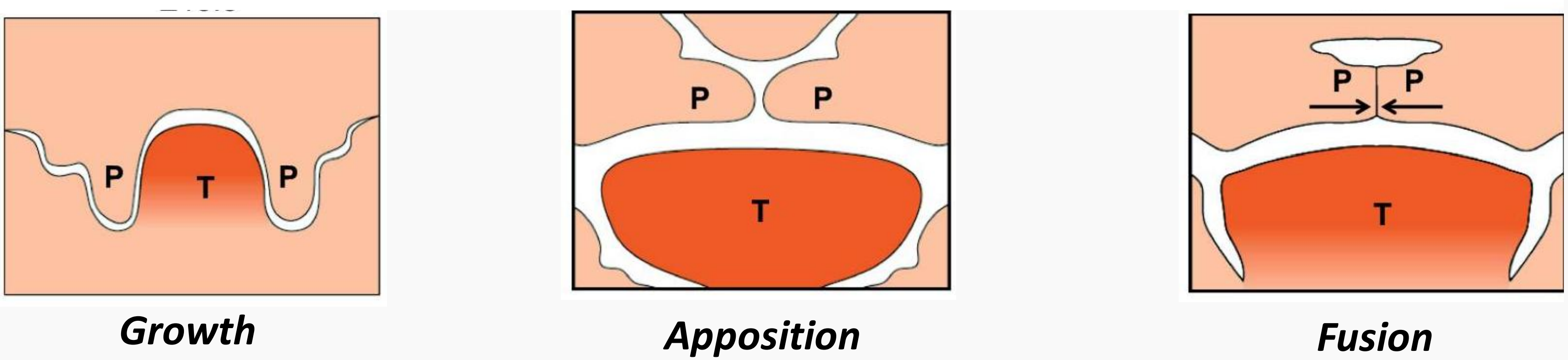
Morphogenetic Fusion

cleft palate affects 1 in 700 births

human

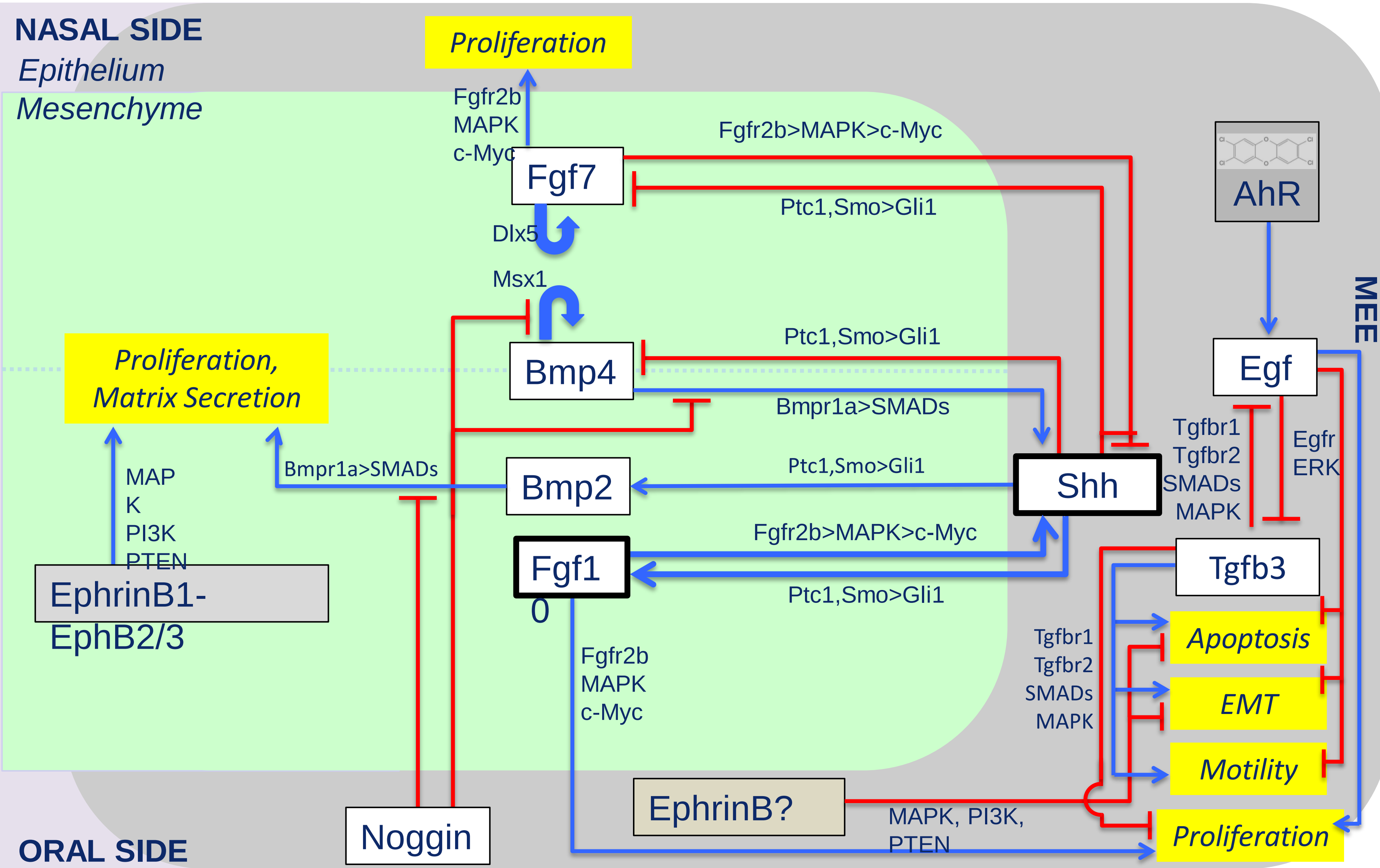


mouse



SOURCE: Dixon (2011) Nature Reviews, Genetics; Ray and Niswander (2012) Development

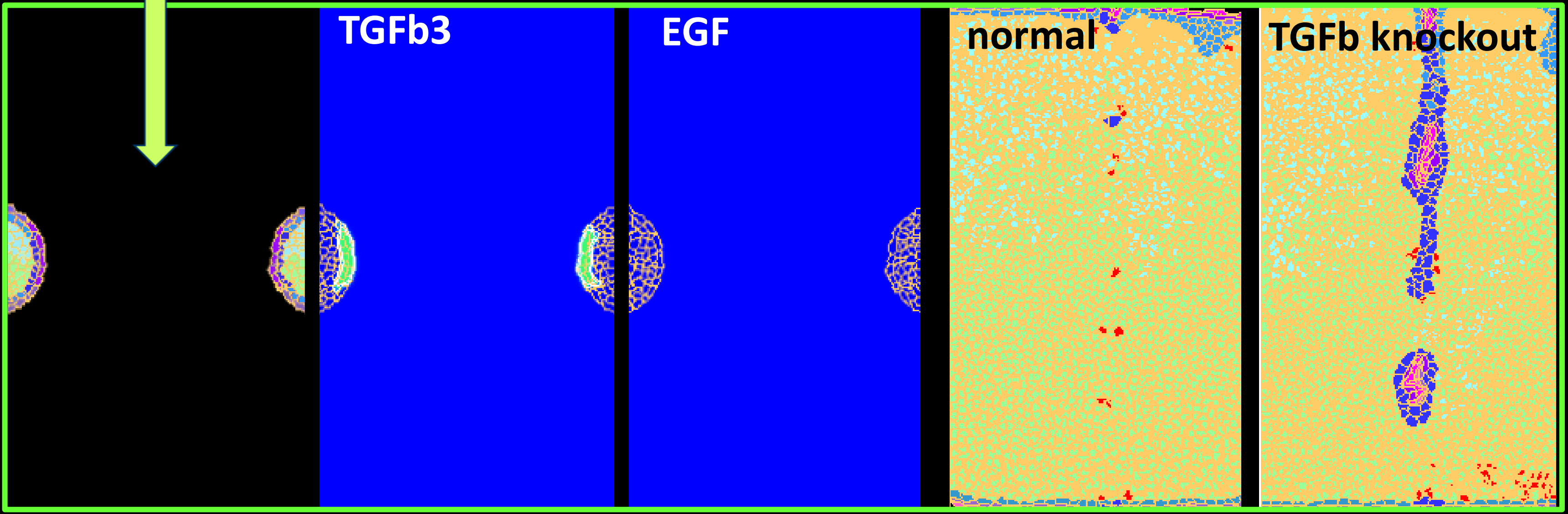
MOUSE PALATAL FUSION: CONTROL NETWORK

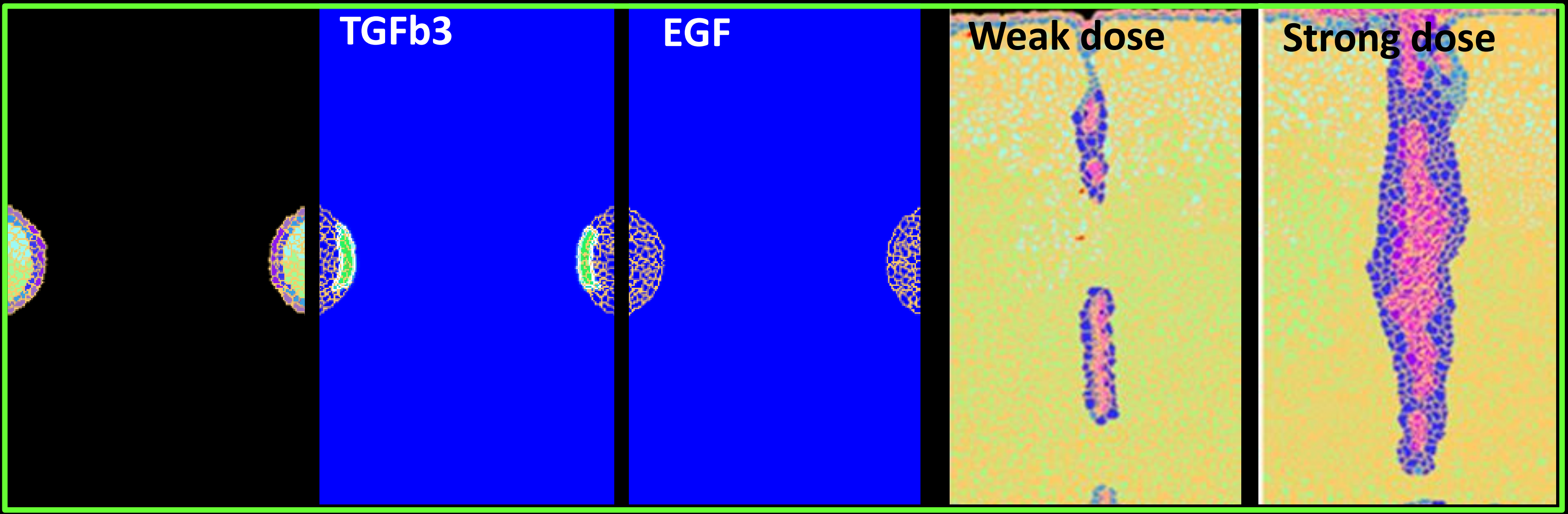


SOURCE: Hutson et al. (in preparation)

Flipping the TGFb3 / EGF switch

KEY EVENT:
seam breakdown





HYPOTHESIS

A computer model that simulates cellular function in the growing embryo can be used to predict the potential impact of chemical exposure during pregnancy and lactation

Executable Biology

Benefits of virtual biology:

- new way to model HTS data
- new tools for quantitative AOP elucidation
- can rapidly sweep many ‘what-if’ scenarios
- hypothesis generation to inform targeted studies

Challenges to design and implementation:

- not a living entity (can only specify rules as we understand them)
- complexity of network structure and dynamics (systems theory)
- finding the sweet-spot to enable but not overspecify performance

Virtual Embryo Team

http://www.epa.gov/ncct/v-Embryo/

Task Management
R Kavlock (ORD), T Bahadori (NPD)
S Darney, E Cohen-Hubal (EPA - CSS)
K Crofton, D Wolf, R Highsmith (MIs)
T Knudsen and S Hunter (Task Leads)

Communications and Outreach
M Linnenbrink (CSS)
M Firestone (EPA - OCHP)
E Mendez (EPA - OCSPP)

Knowledge Management Systems
R Spencer, N Baker, T Transue, T Cathy, M Brown (LHM), A Singh (Syngenta Corp)

Early Lifestage Exposure and Dosimetry
H El-Masri, L Adams (EPA-NHEERL)
J Kenneke, S Marchitti, C Mazur (EPA-NERL)
I Shah, J Wambaugh (EPA-NCCT, CSS 2.2.1)

Predictive Signatures (ToxCast)
N Sipes, M Martin, R Judson, A Richard, K Houck (EPA-NCCT)
W Mundy, T Shafer (EPA-NHEERL)

Vascular Development
N Kleinstreuer, J Franzosa (EPA-NCCT)
S Padilla, T Tal, K Jensen, J Olin (EPA-NHEERL)
M Hemmer, K Nelson, S Vickery, P Harris (EPA-GED)
M Bondesson, C McCollum (TIVS – U Houston)
S Clendenon, A Shirinifard (TIVS – Indiana U)
E Carney, R Ellis-Hutchings, Raja Settivari (DOW)
T Heinonen, R Sarkanen (FICAM)

mESC Differentiation
S Hunter, K Chandler, M Rosen, W LeFew, H Nichols, S Jeffay, M Hoopes, J Royland, A Tenant (EPA-NHEERL)
R Cabrera, R Finnell (TIVS – U Texas)

Modeling Dysmorphogenesis
T Knudsen, M Rountree, W Setzer, M Leung (EPA-NCCT)
R Dewoskin (EPA-NCEA)
C Lau, B Abbott, C Wolf, M Narotsky (EPA-NHEERL)
S Jeyaraman, J Glazier, M Swat (TIVS – Indiana U)
S Hutson (Vanderbilt U)