United States
Environmental Protection
Agency

Evolution

~~Revolution~~ in Toxicity Testing and Risk Prediction for Chemicals in the Environment

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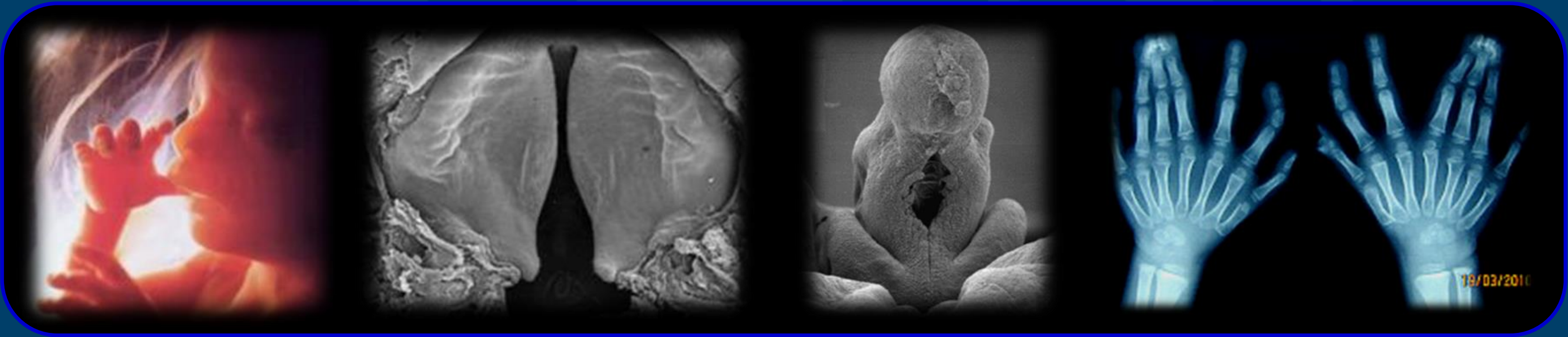

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American Society of Andrology, Atlanta GA, April 8, 2014

Problem Statement

- ~83,000 chemicals: too many to test with standard animal-based methods due to cost, time, and animal welfare considerations;
- high-throughput screening (HTS) paradigm: *in vitro* data and *in silico* models for hazard prediction;
- high-throughput exposure (HTE) models: integrate chemical properties, production and use, reverse-dosimetry;
- prioritize chemicals for targeted testing: Adverse Outcome Pathways (AOPs) for human health and ecological relevance;
- how can we evolve emerging principles in *predictive toxicology* to promote children's environmental health (CEH) protection?

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

CSS Task Management
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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)

Communications and Outreach
M Linnenbrink (CSS, NCCT)
M Firestone (EPA - OCHP)
S Darney (EPA – CEH roadmap)

Knowledge Management Systems
R Spencer, T Cathy, T Transue, M Brown (LHM-EMVL)
N Baker (LHM)

Early Lifestage Exposure and Dosimetry
H El-Masri, L Adams (EPA-NHEERL)
J Kenneke, S Marchitti, C Mazur (EPA-NERL)
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v-Embryo™

Predictive Signatures (ToxCast)
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S Padilla, T Tal, K Jensen, J Olin (EPA-NHEERL)
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S Clendenon, A Shirinifard (TIVS – Indiana U)
E Carney, R Ellis-Hutchings, Raja Settivari (DOW)
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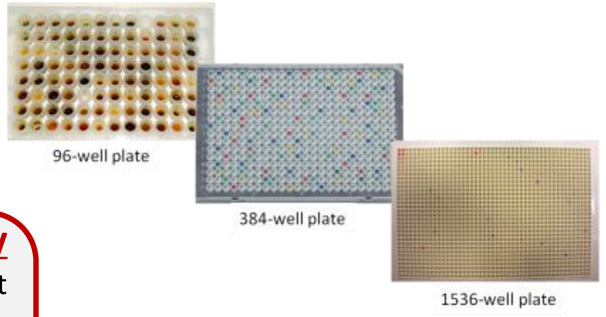
S Jeffay, M Hoopes, J Royland, A Tenant (EPA-NHEERL)
R Cabrera, R Finnell (TIVS – U Texas)

Modeling Dysmorphogenesis
T Knudsen, W Setzer, M Leung, B Ahir (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)

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 (2014).
  - **Phase-IIIa:** adds 1001 compounds in a subset of ~100 assays (2014 -); E1K adds 880 chemicals in ~50 endocrine-related assays.
- **Tox21:** partnership of federal agencies.
  - 8193 chemicals in dozens of HTS assays (ongoing)
  - brings total number of chemicals to ~10,000

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

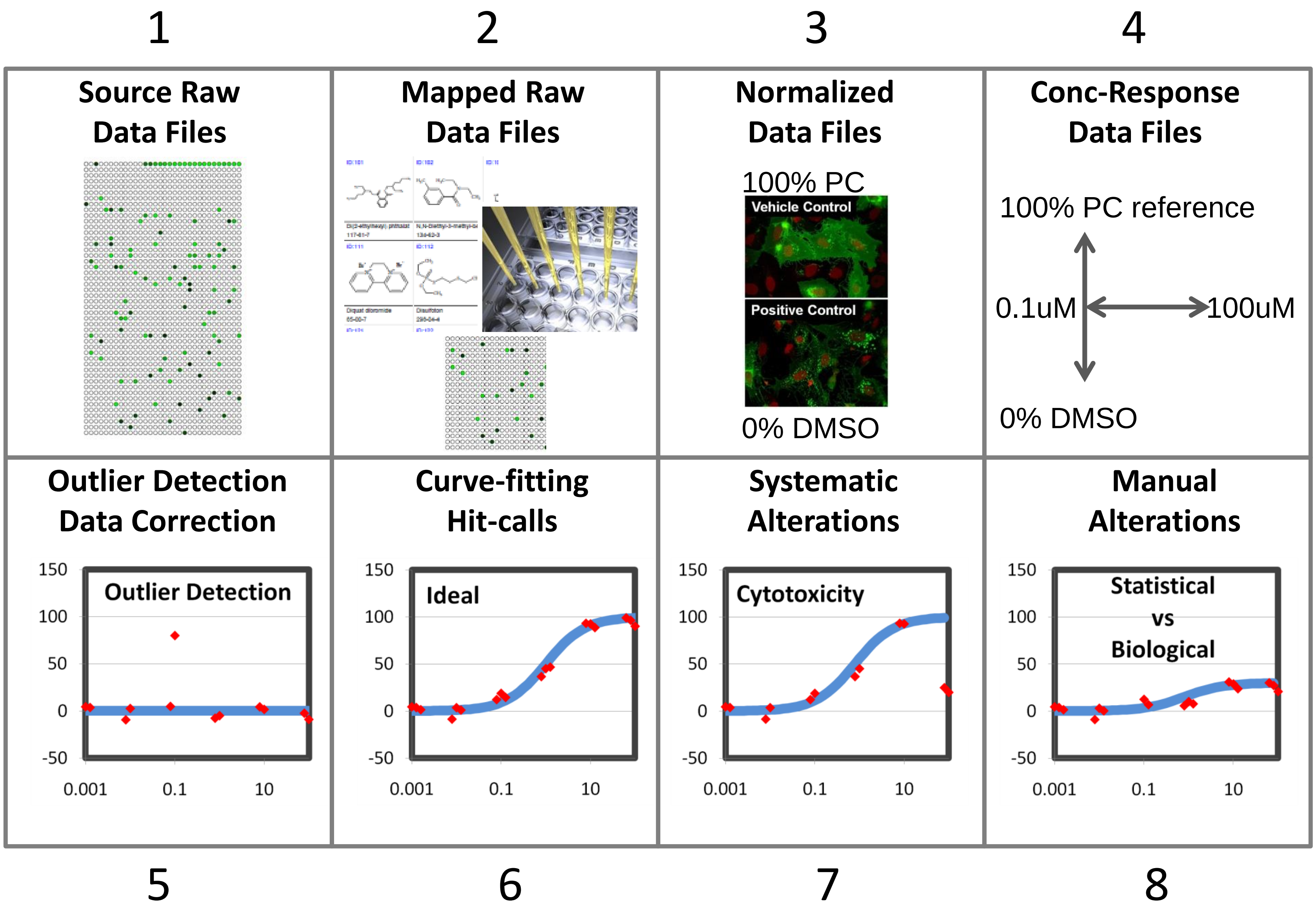
Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

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

DevTox (new)
ES cell secretome
neuroprogenitors
morphogenesis
differentiation

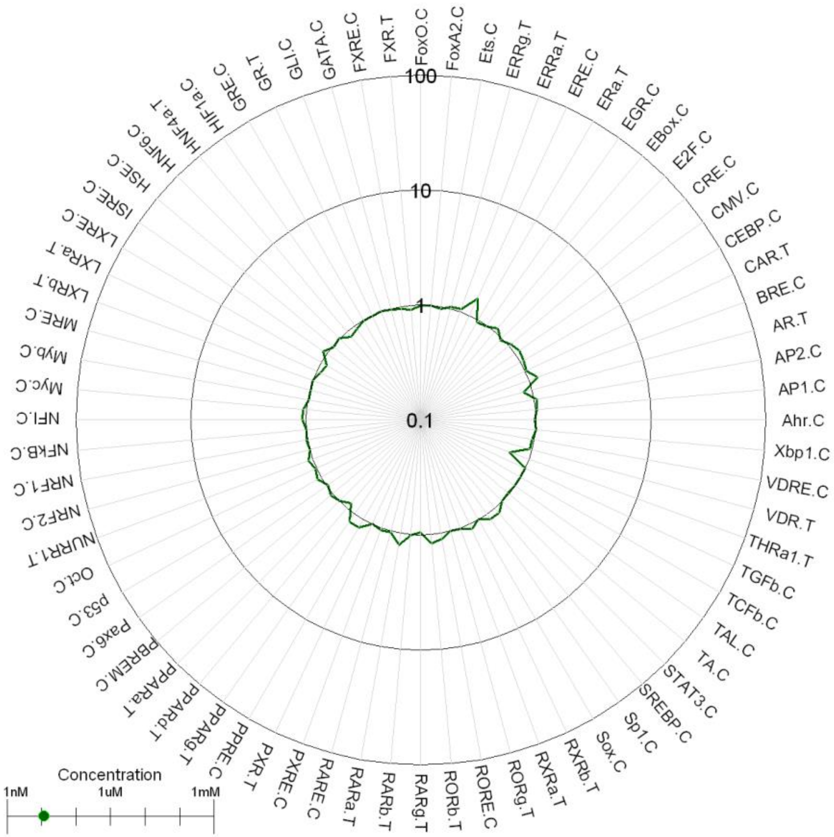
List of assays and related information at: **http://www.epa.gov/ncct/**

ToxCast Data Analysis Pipeline



SOURCE: M Martin, NCCT

EXAMPLE: reporter gene activation (Attagene)



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

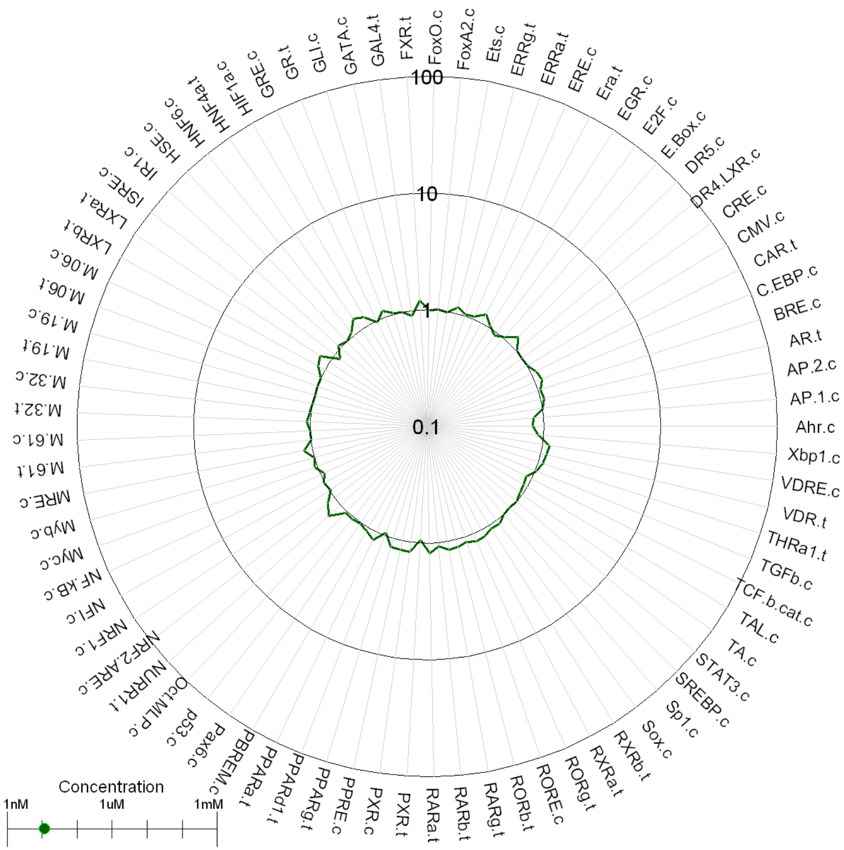
Animation shows concentration response

Example: a pharmaceutical tested in ToxCast Phase II

Sunburst: loss of specificity

SOURCE: M Martin, NCCT

EXAMPLE: reporter gene activation (Attagene)



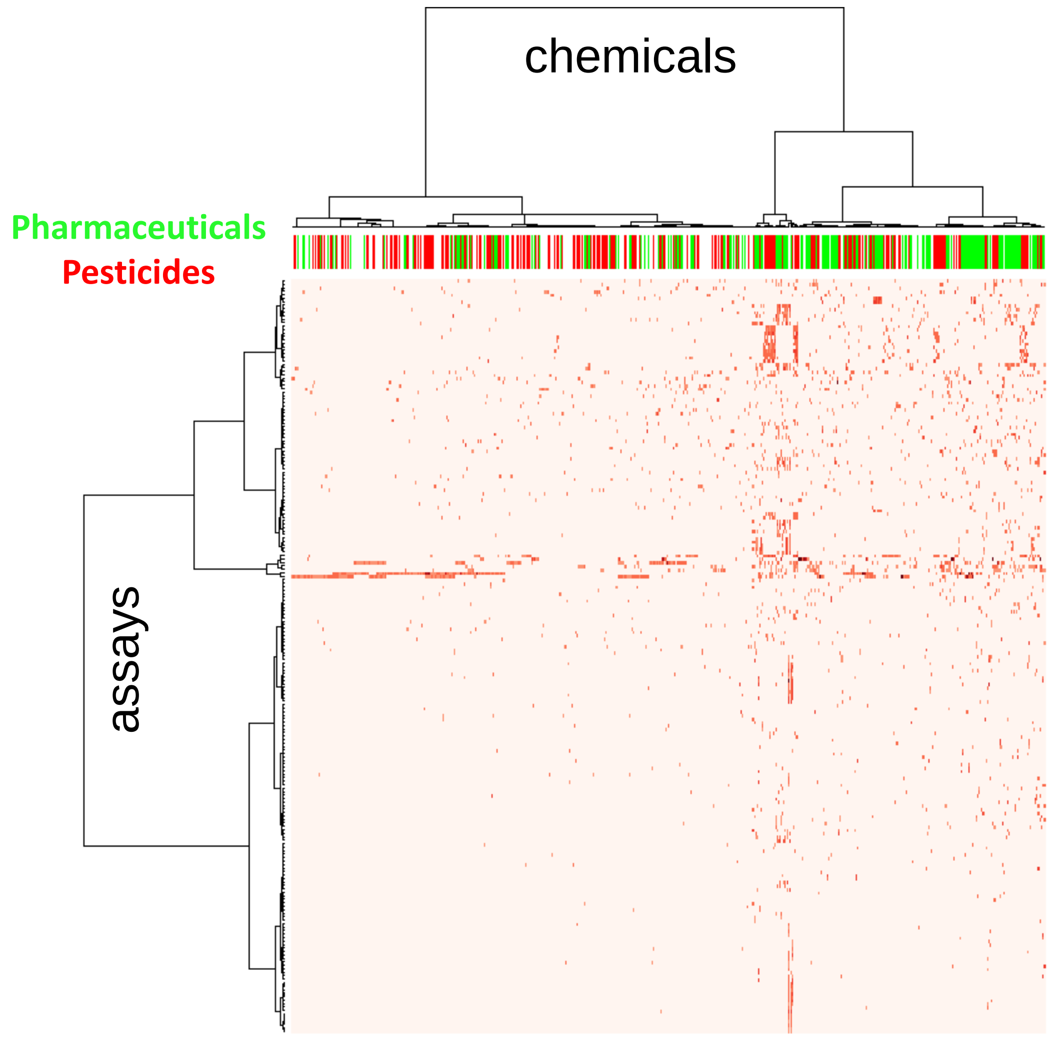
Spider plot maps the activity of 72 reporter gene pathways tested in ToxCast

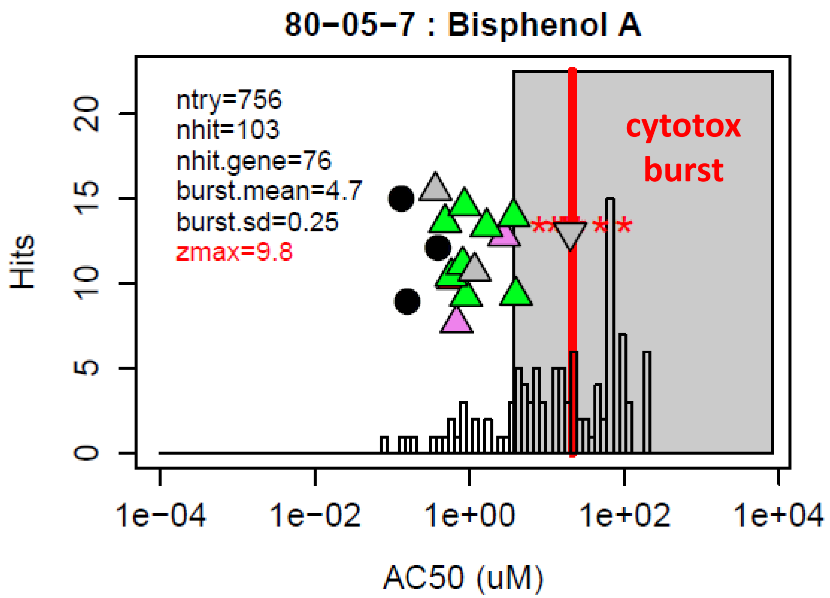
Example: concentration response for an environmental chemical (Pentachlorophenol)

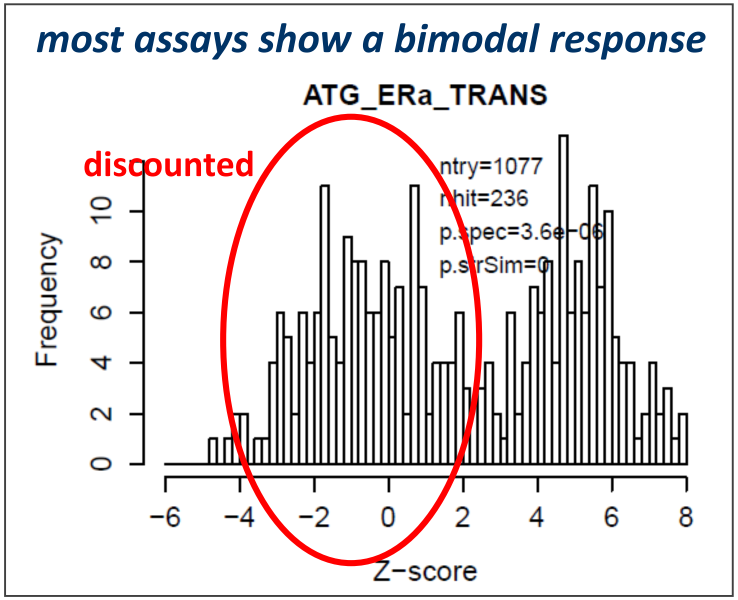
Sunburst: approaching cytotoxicity

SOURCE: M Martin, NCCT

ToxCast-II: 1051 Chemicals x 790 Assay Readouts (gene function)







SOURCE: R Judson, NCCT

iCSS Dashboard: public delivery portal for ToxCast

Home | Export

EPA has released the first beta version (version 0.5) of the **Interactive Chemical Safety for Sustainability (iCSS) Dashboard**. The beta version of the iCSS Dashboard provides an interactive tool to explore rapid, automated (or in vitro high-throughput) chemical screening data generated by the Toxicity Forecaster (ToxCast) project and the federal Toxicity Testing in the 21st century (Tox21) collaboration.

Please email the Dashboards Team to provide feedback on ways to improve the Dashboard or to be added to a mailing list to receive status updates. The intent is to use stakeholder feedback to develop updated versions of the iCSS Dashboard and to add more Dashboards.

To get the best possible experience using the CSS Dashboard application we recommend using Mozilla Firefox or Google Chrome.

Users of the iCSS Dashboard v0.5 can perform basic data and chemical selection, as well as simple data exploration in a seamless environment. EPA will continuously add functionality and improve overall usability and performance. The initial release conveys the conceptual framework and design of the iCSS web application.

The iCSS Dashboard contains the results from more than 800 Assay Endpoints (High-Throughput Screening-HTS Data) across over 1,800 chemicals from seven primary HTS assay sources. The release of the iCSS Dashboard coincides with the release of the ToxCast Phase II data. All of the ToxCast Phase II data (including assay summary activity files, assay description files, effect and endpoint data files from animal toxicity studies, concentration response data files & chemical library and structure files) are available on the ToxCast Data Download Page.

The vision is for the Dashboard to evolve into an iCSS web application which will become the portal to access all EPA computational toxicology research data and studies including:

- Rapid, automated (or in vitro high-throughput) chemical screening data generated by the EPA's Toxicity Forecaster (ToxCast) project and the federal Toxicity Testing in the 21st century (Tox21) collaboration.
- Aggregated public sources of chemical toxicity data (AcTOR).
- Animal toxicity studies (ToxRefDB). Chemical exposure data and prediction models (ExpoCastDB).
- High quality chemical structures and annotations (DSSTox).

ToxCast Data Use Considerations

- The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision context.
- Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve.

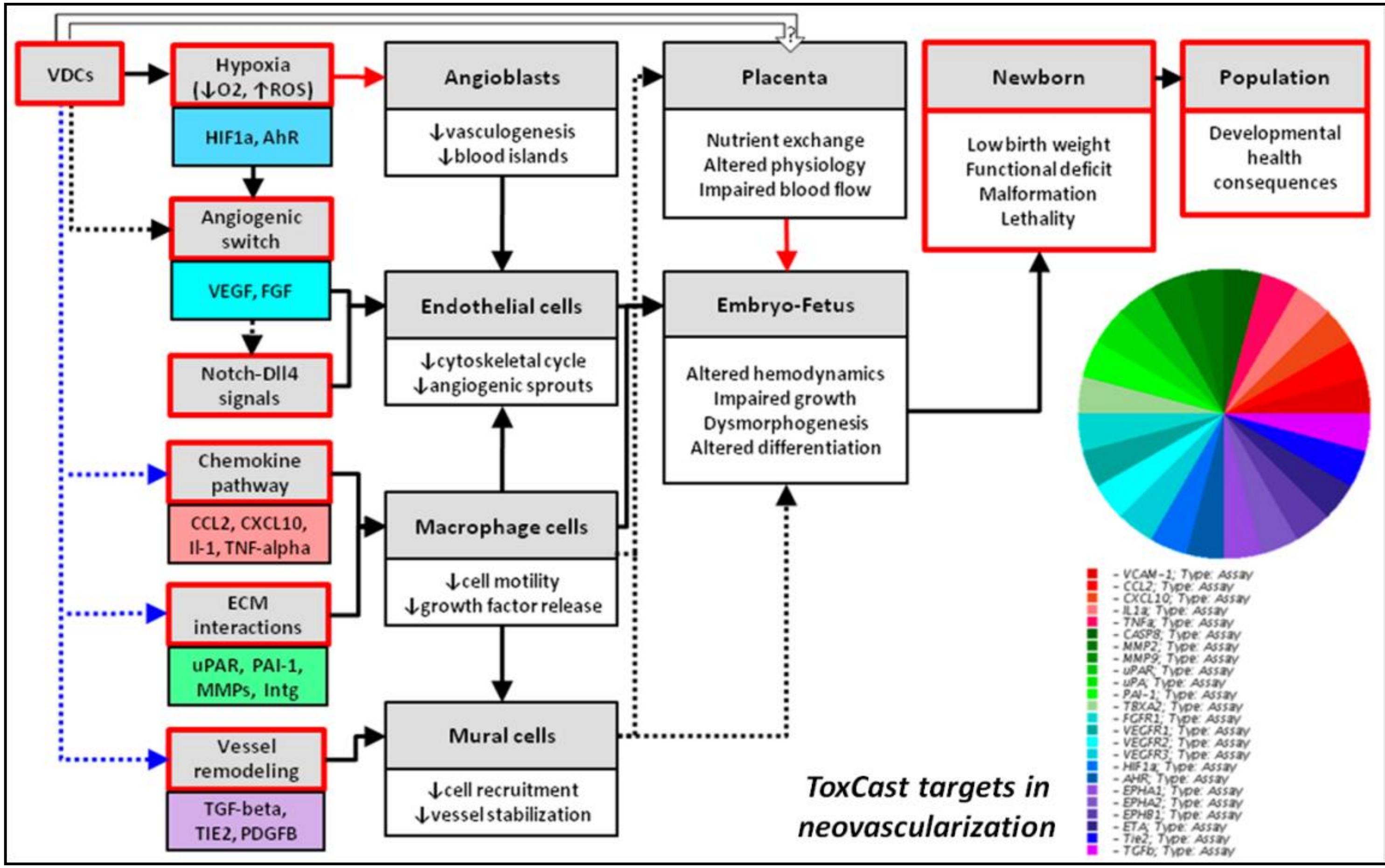
Stakeholder Opportunities to Learn & Explore the Data

- Data Challenges
- Stakeholder Workshops & Data Summit

http://actor.epa.gov/dashboard/

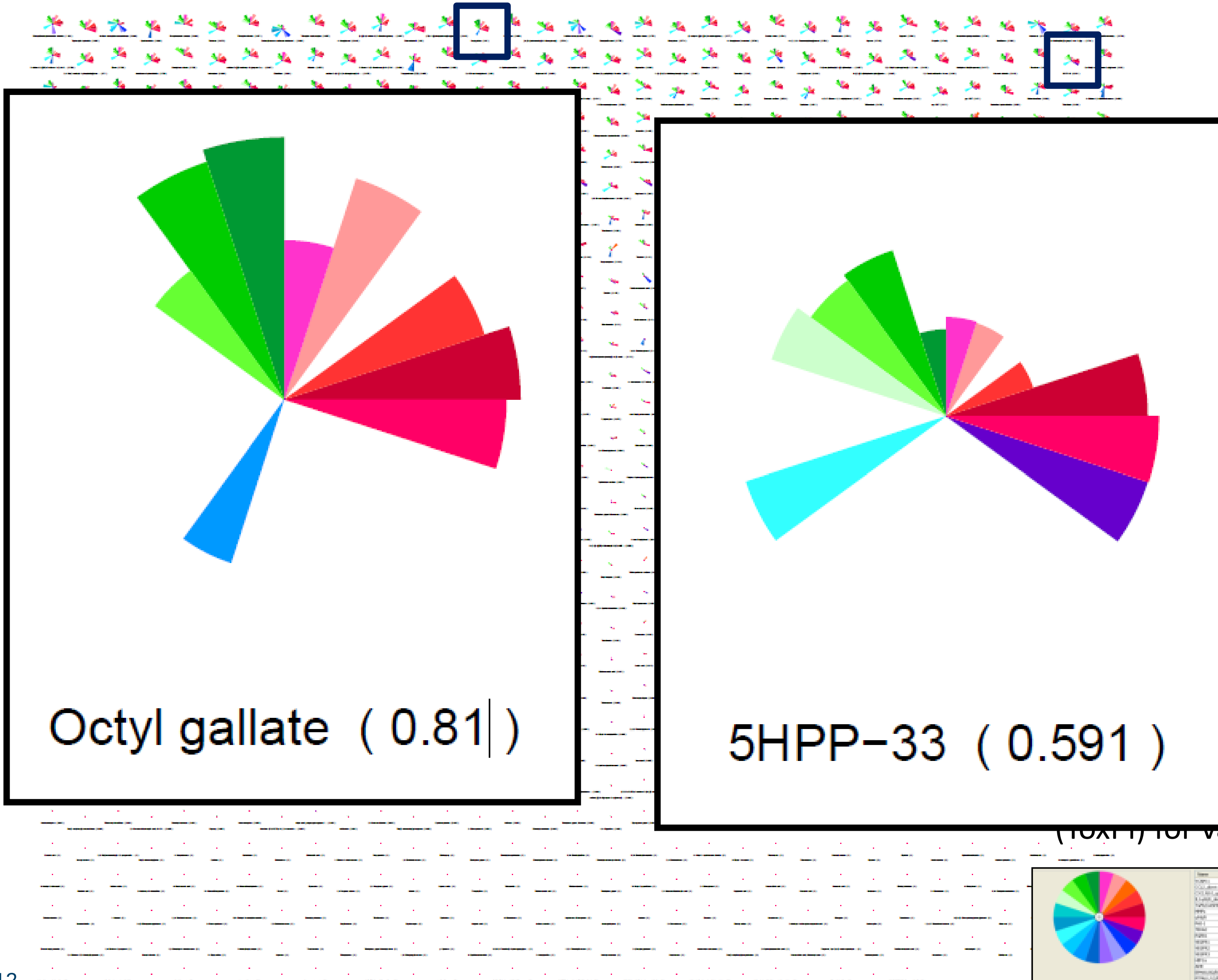
SOURCE: M Martin, NCCT

AOP FRAMEWORK: embryonic vascular disruption

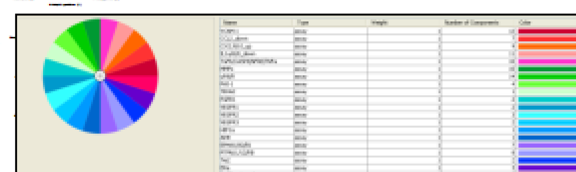


SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res. C 93: 312-323.
SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol 9(4): e1002996.

1060 CHEMICALS IN TOXCAST-II RANKED BY pVDC SCORE



…oritization Index
(ToxPi) for vascular disruption

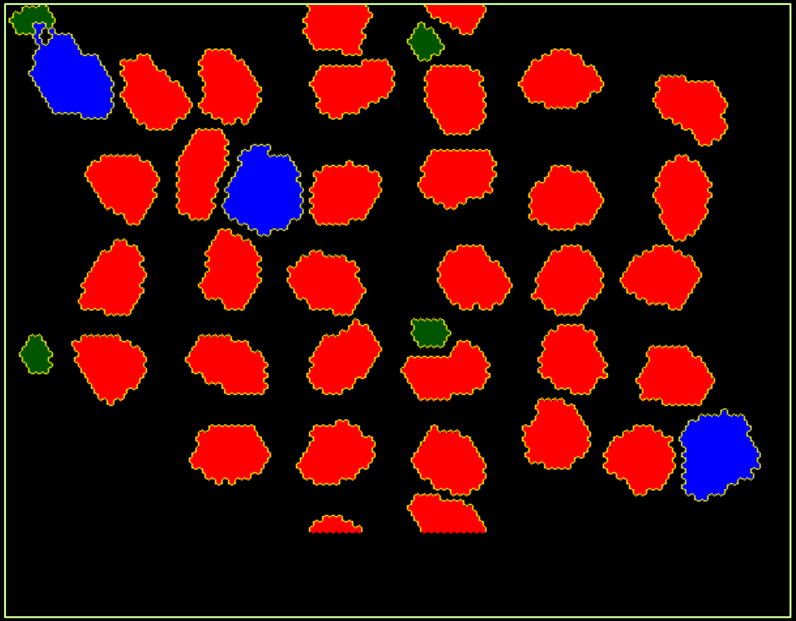


CELL AGENT-BASED MODELS (ABMs)

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

In vitro

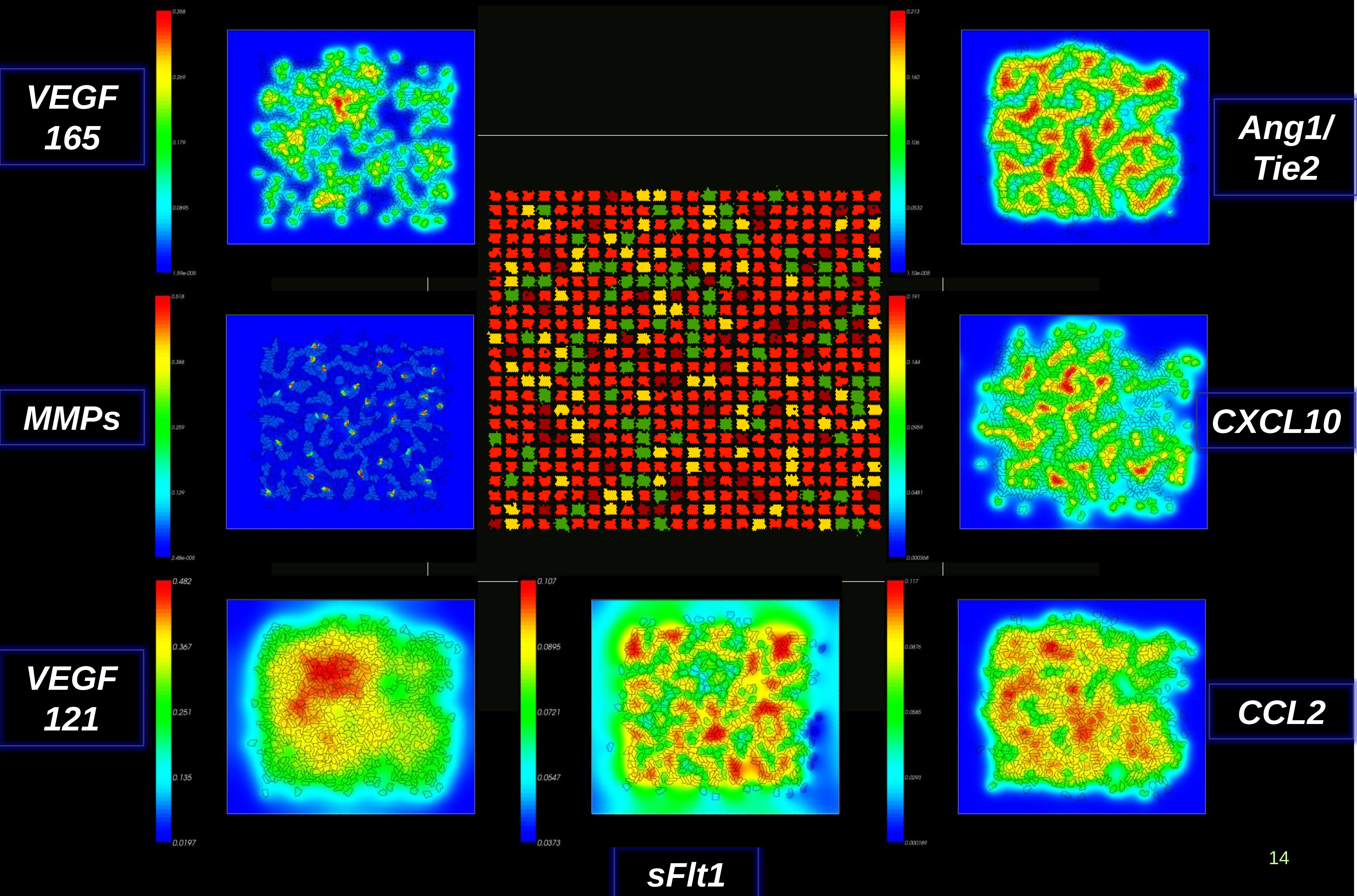
In silico



SOFTWARE: www.CompuCell3D.org
James Glazier and colleagues, Indiana U

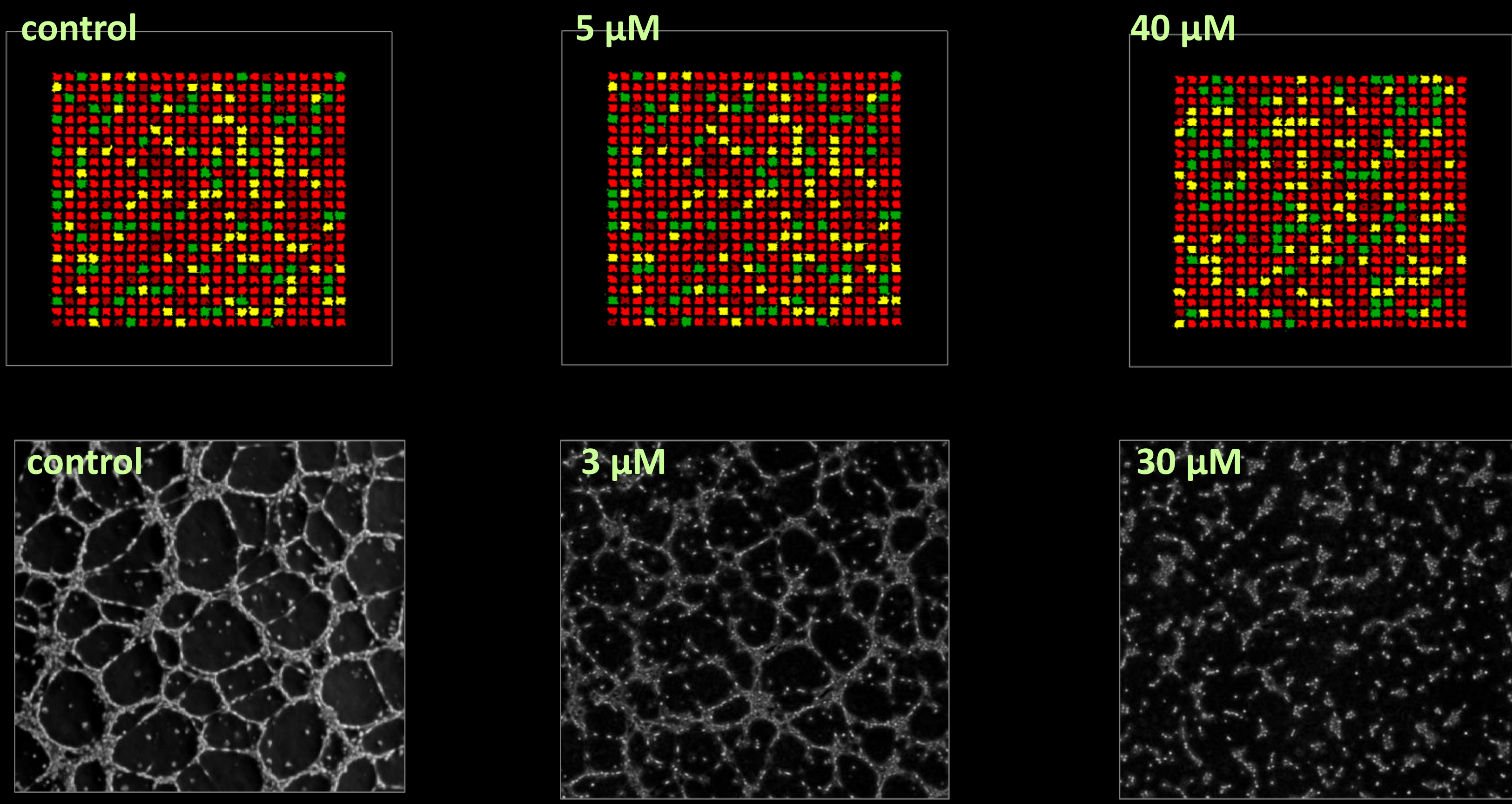
macrophage
RBC
bug

CELLULAR ABM FOR ENDOTHELIAL NETWORK



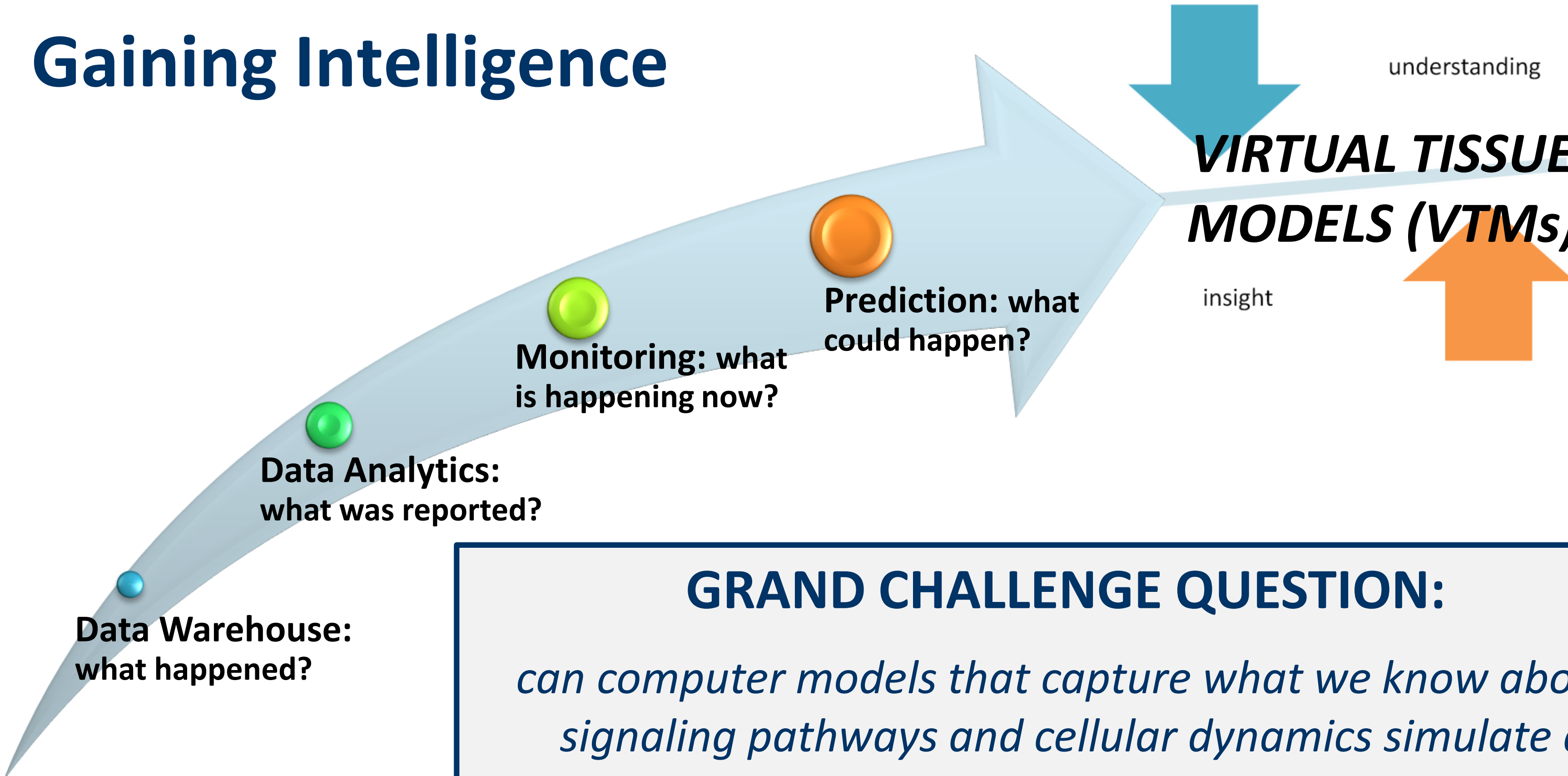
CELLULAR ABM PERTURBED WITH HTS DATA

- simulation perturbed with ToxCast-II data for 5HPP-33 captures the anti-angiogenic effect and concentration-response



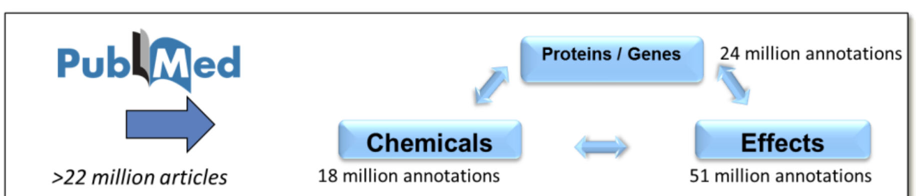
SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol 9(4): e1002996. doi:10.1371/journal.pcbi.1002996.

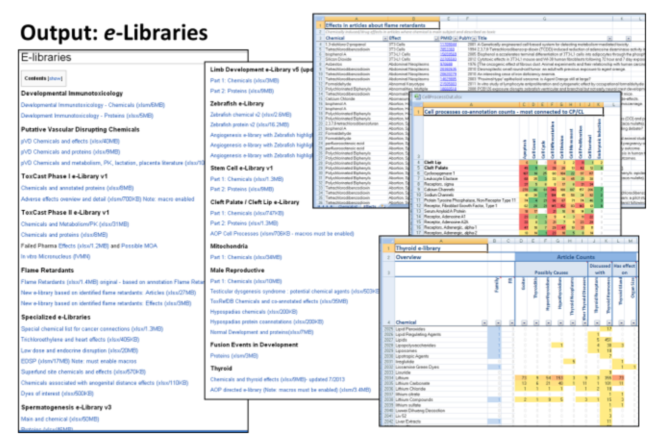
Gaining Intelligence



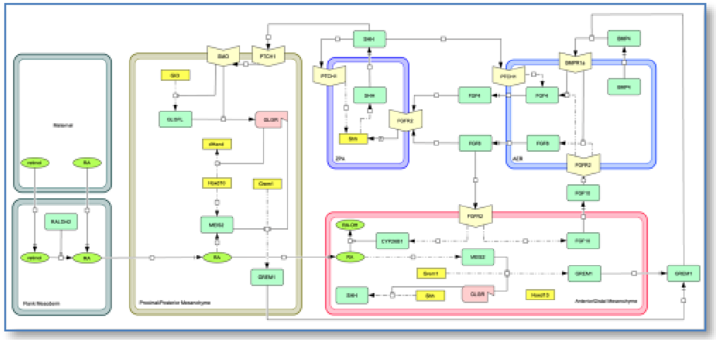
GRAND CHALLENGE QUESTION:

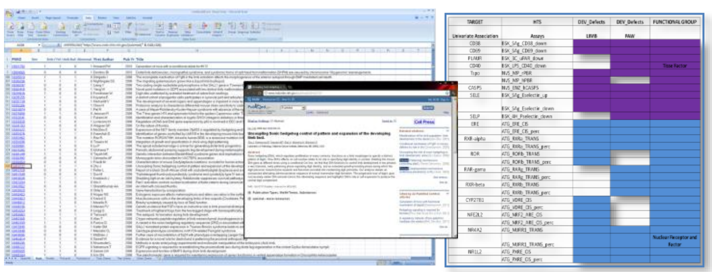
can computer models that capture what we know about signaling pathways and cellular dynamics simulate and predict performance of a biological complex system?

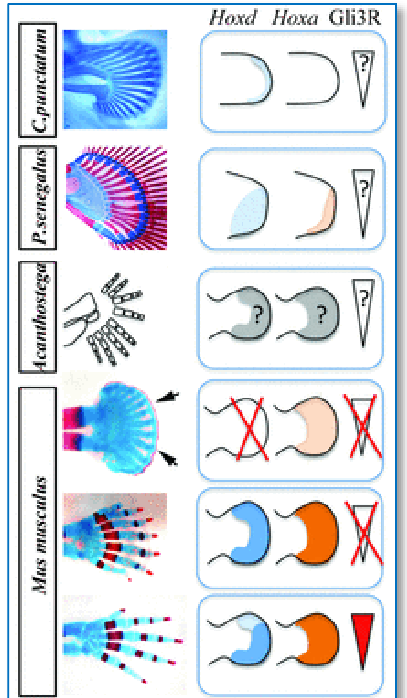




LIMB DEVELOPMENT and PREDICTIVE MODELING







Sheth et al. (2012) Science 338: 1476

Science 338 (2012)
(with permission from J Sharpe)

Data Mining

What pathways are relevant to dysmorphogenesis? (AOPs)

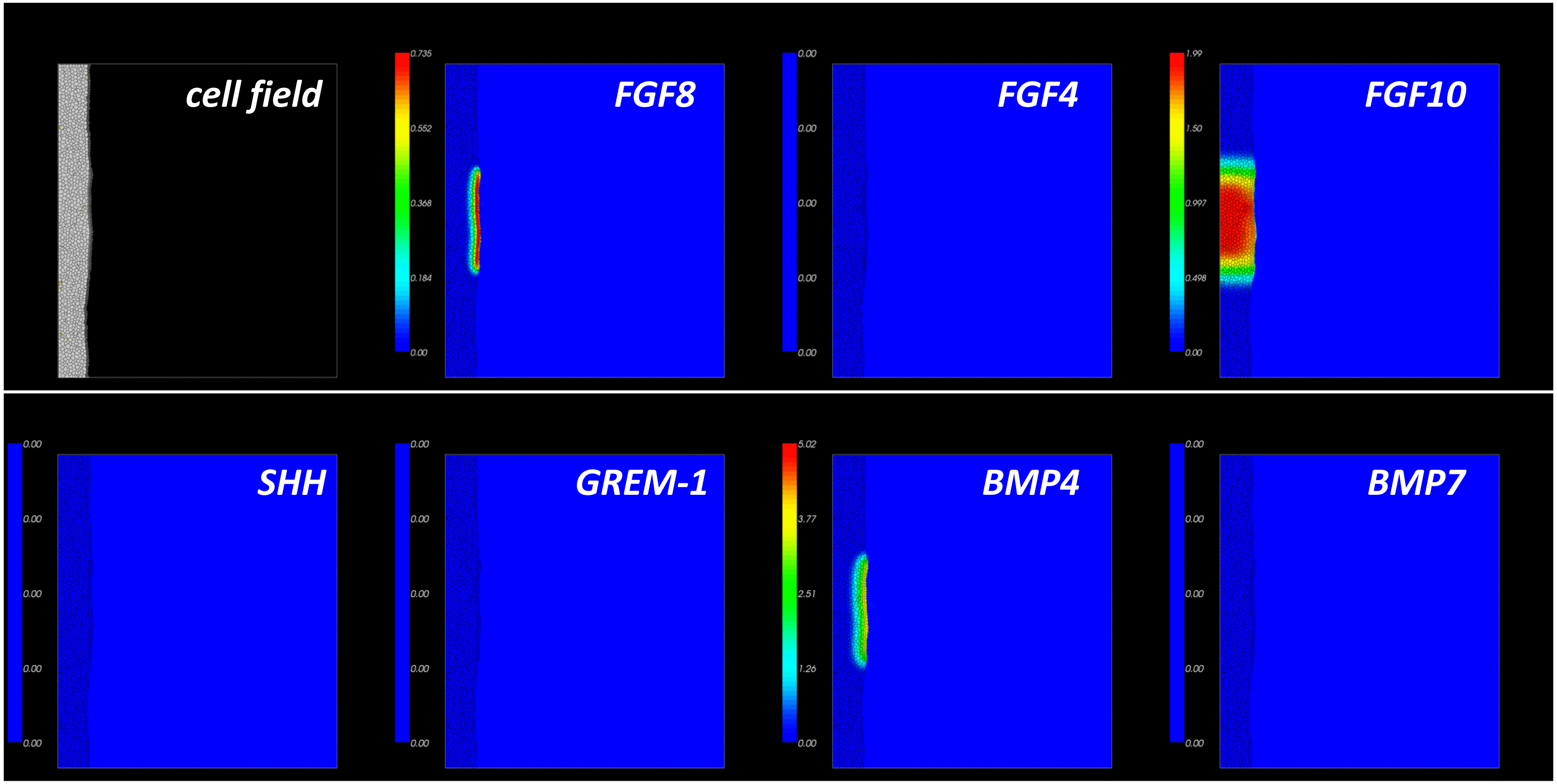
Systems Model

How can we model the underlying biology and cellular dynamics? (VTMs)

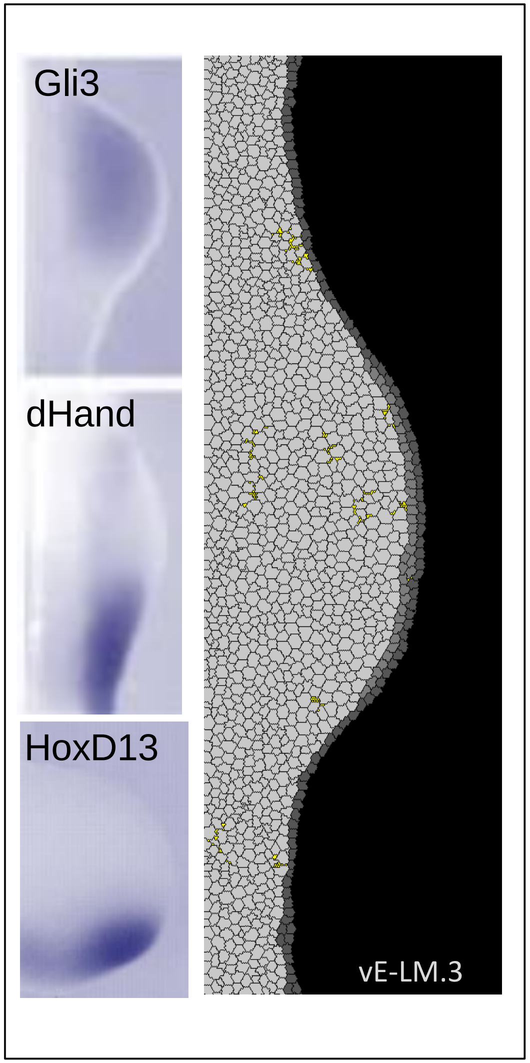
Limb/Paw/Digital Defects

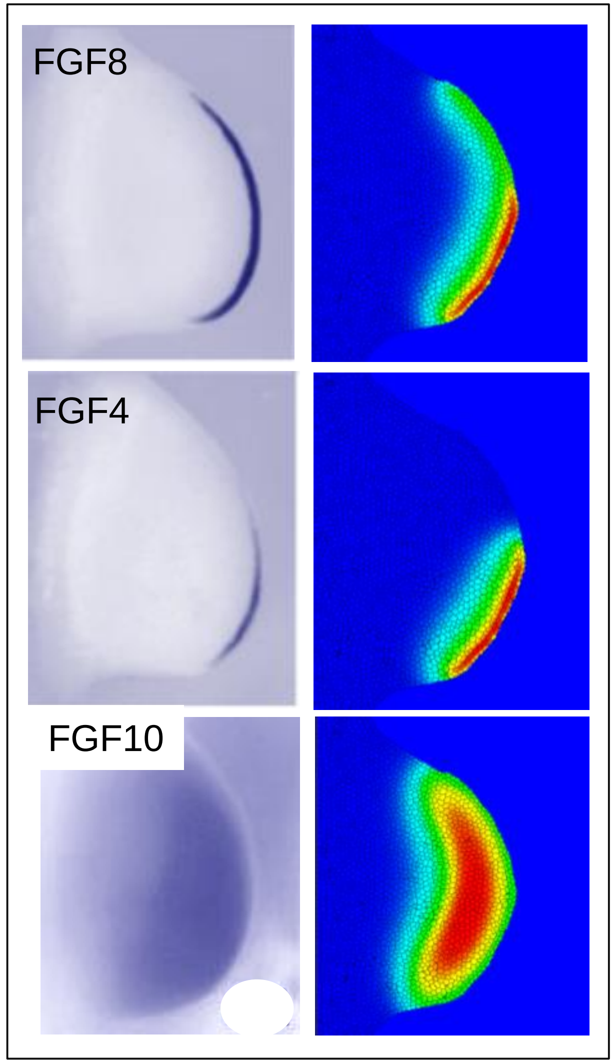
How can VTMs that recapitulate spatio-temporal events enhance mechanistic understanding of AOPs and quantitative predictivity?

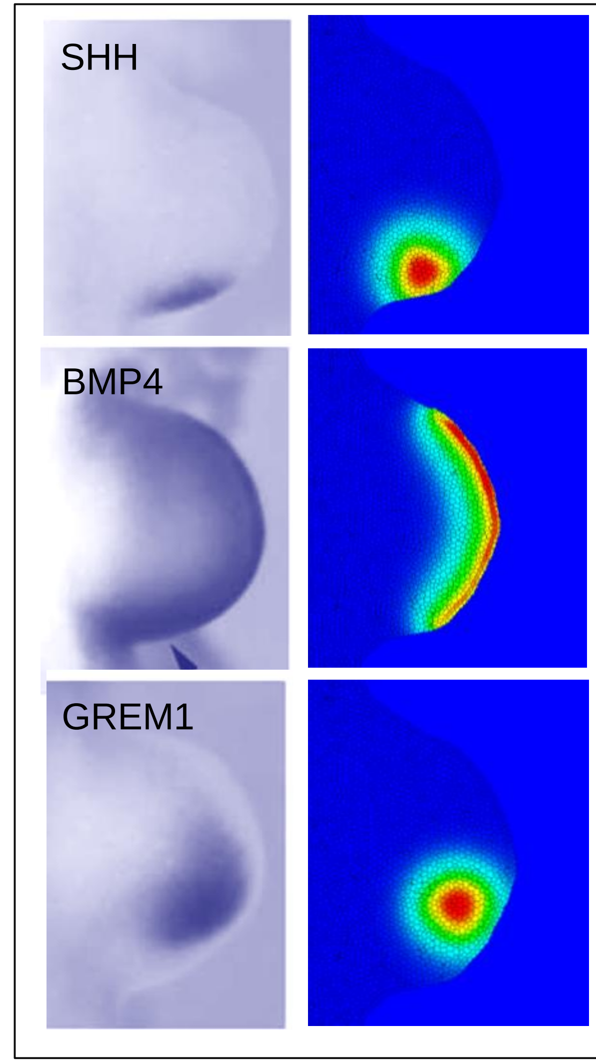
LIMB-BUD OUTGROWTH: CONTROL SIMULATION



SOURCE: Knudsen et al. (manuscript in preparation).







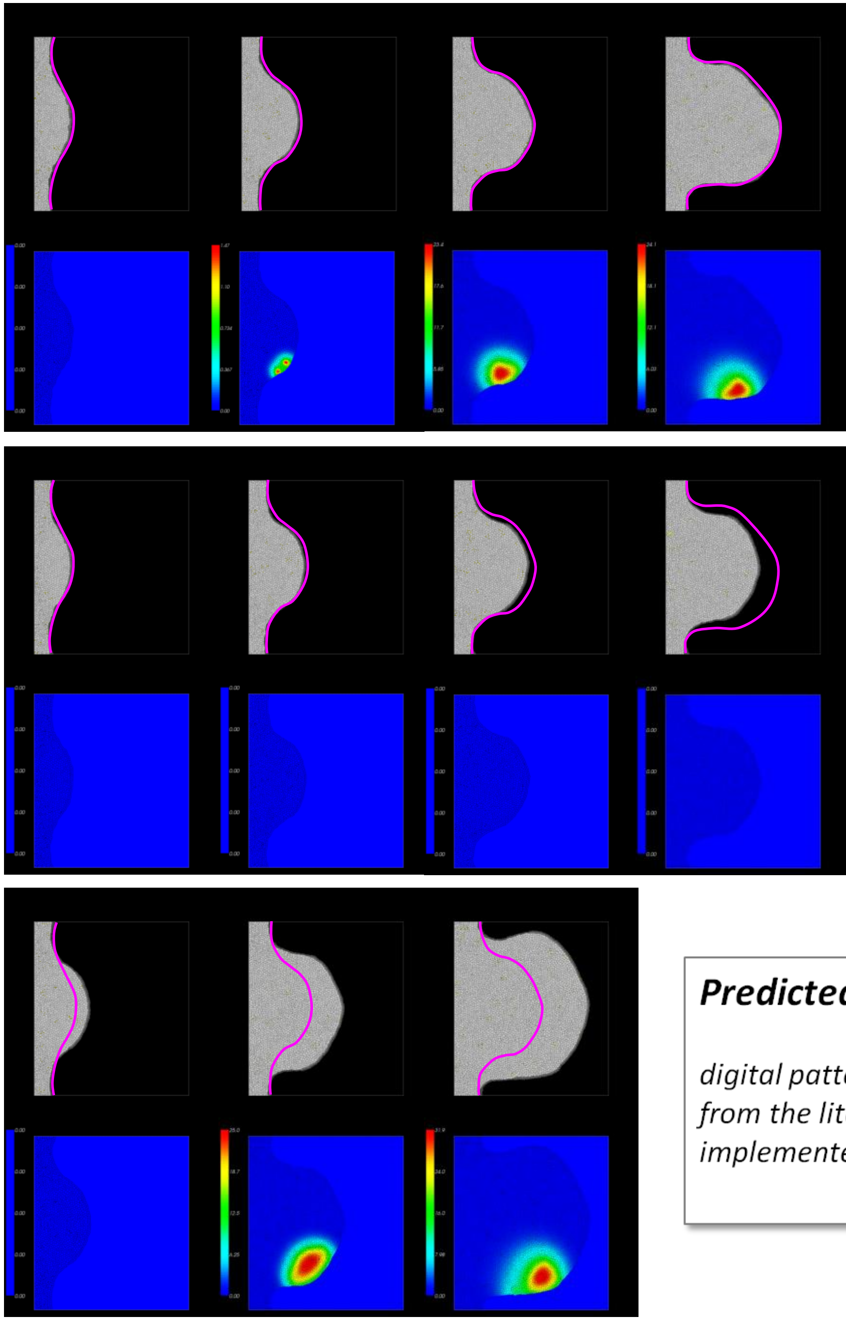
ISH (mouse literature) vs ABM

Simulated outgrowth

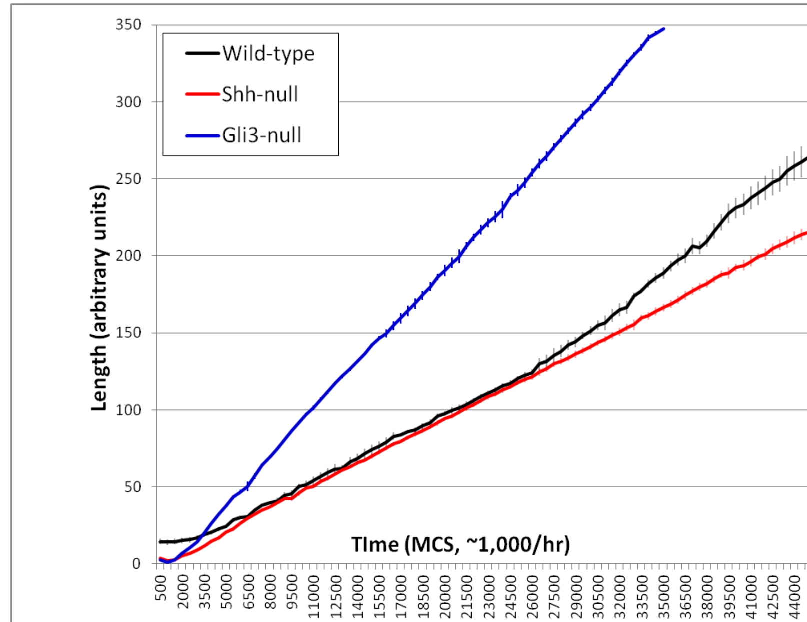
Wild-type

Shh-null

Gli3-null



Rate of elongation (n=5)

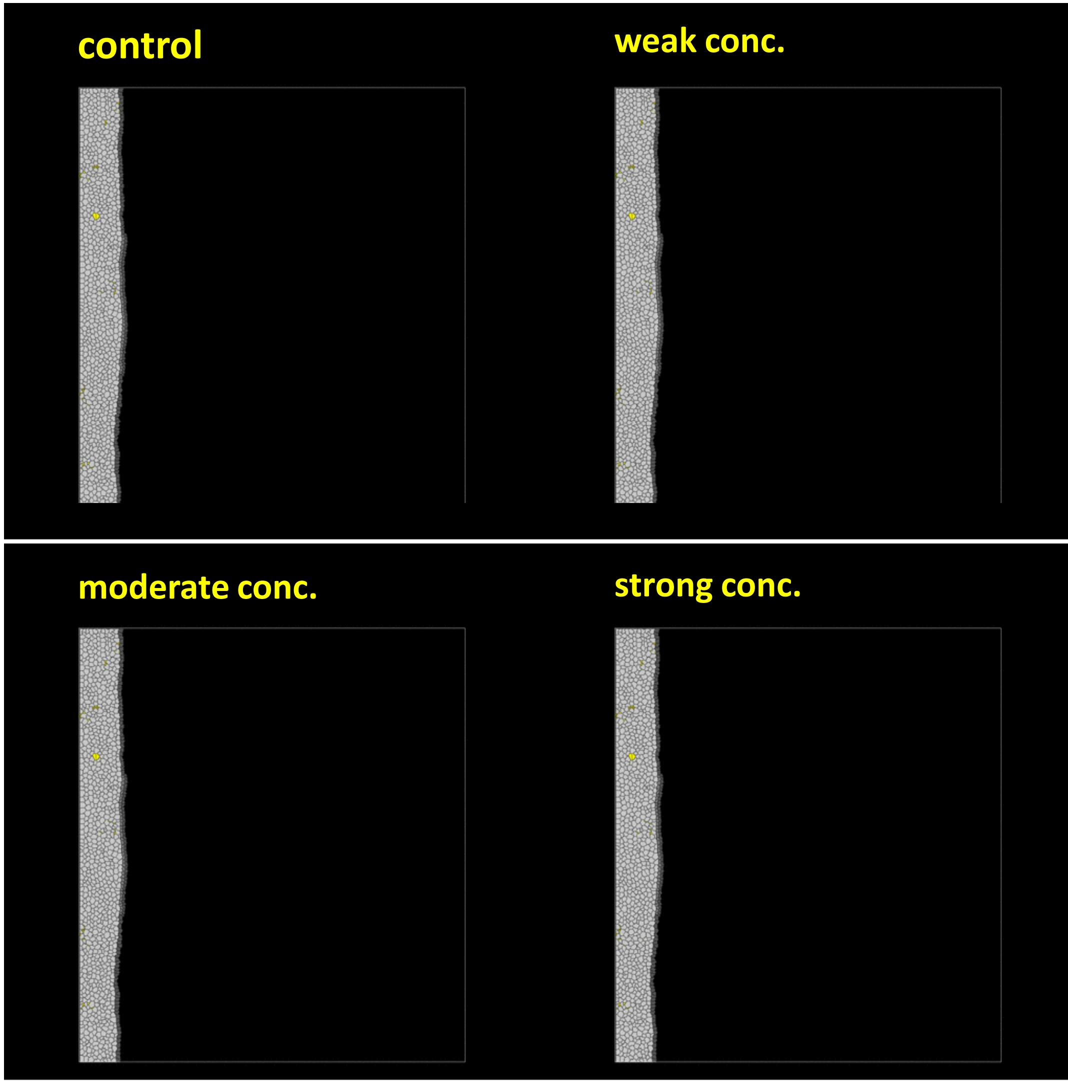


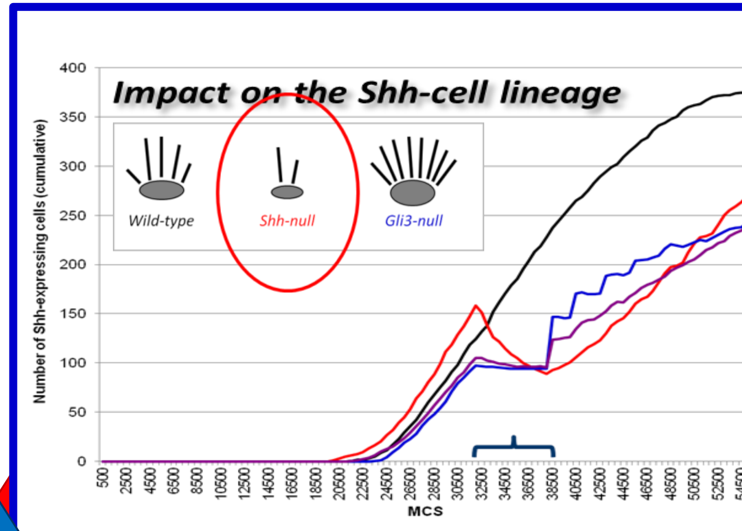
Predicted outcomes

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

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

‘What-If?’ - DISRUPTION OF CELL GROWTH



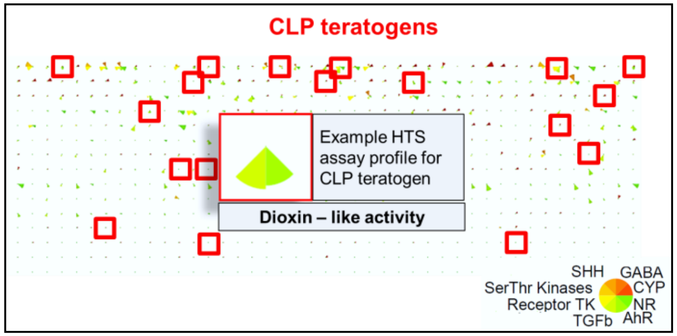


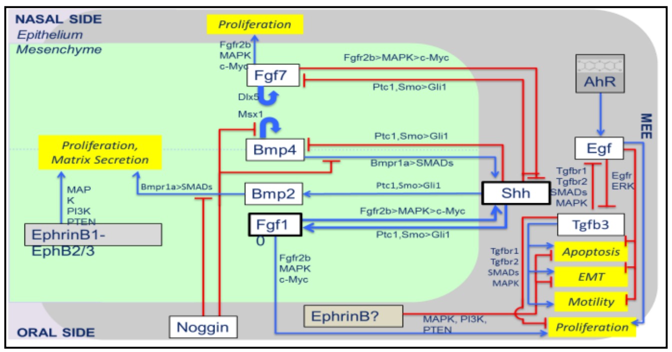
“At term, hindlimb defects (lateral digit agenesis) were observed at low frequency at doses of 30 mg/kg and with higher incidence and severity at 35 and 40 mg/kg …” –

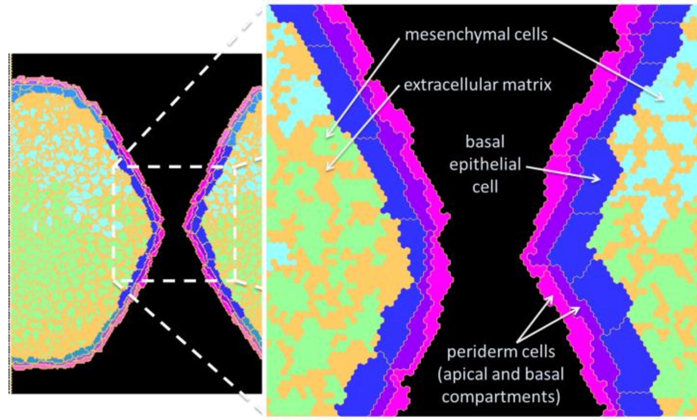
Shuey et al. 1994, Teratology 50: 379–386

… and predict impact on embryo.

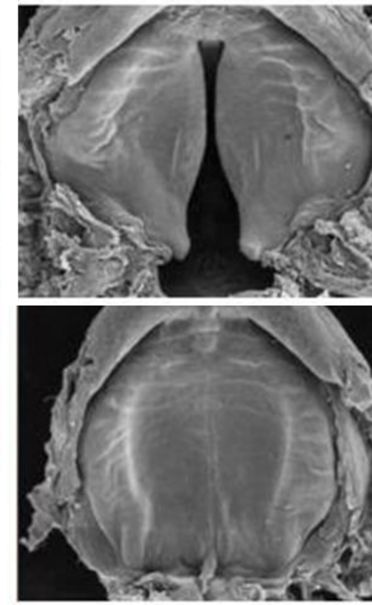
VIRTUAL PALATE







Modeling Morphogenetic Fusion



Palatal Closure Defects

Data Mining

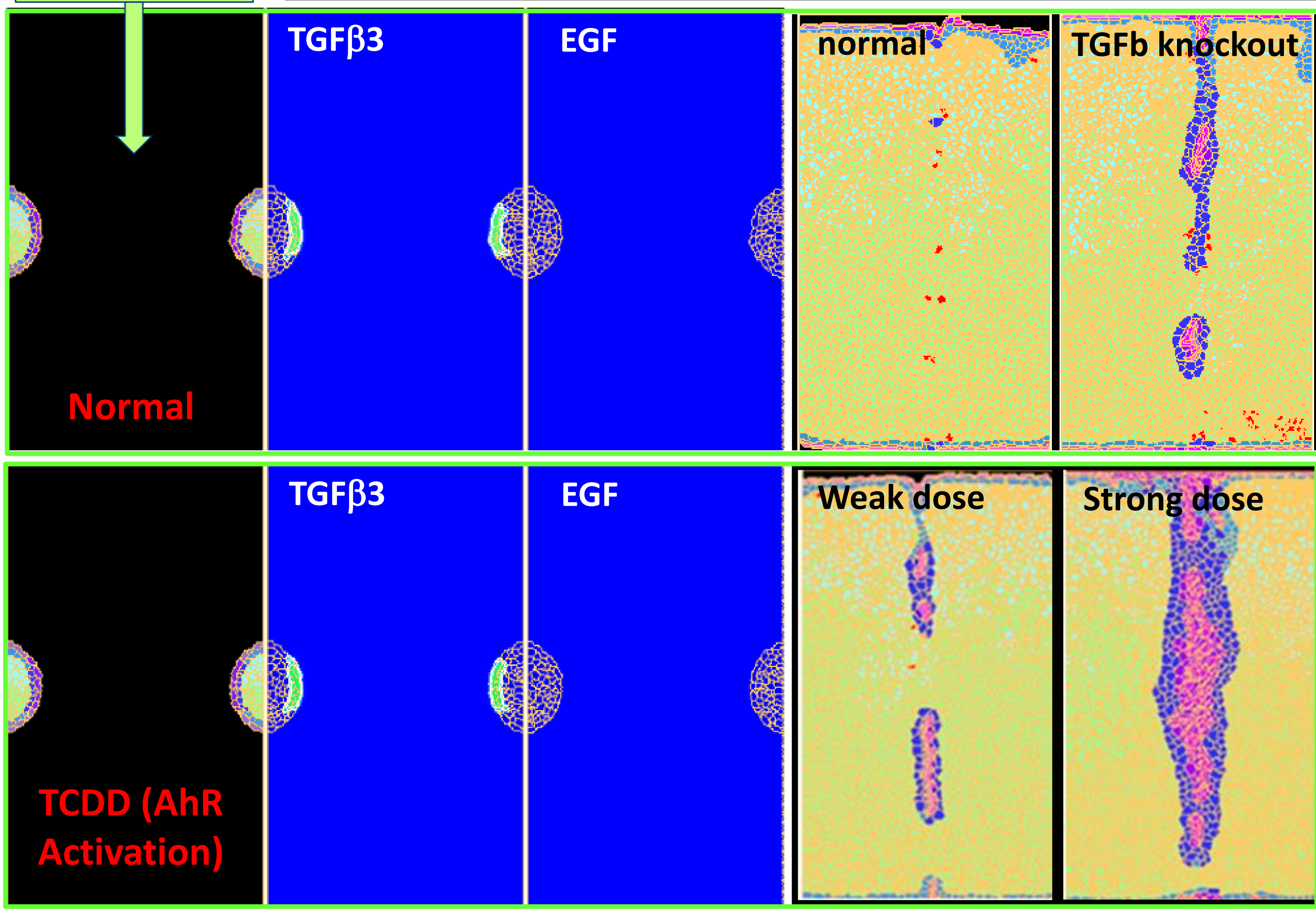
What pathways are relevant to cleft palate? (AOPs)

How can we model the underlying biology and cellular dynamics of morphogenetic fusion? (VTMs)

How do AhR-responsive pathways influence specific cellular behaviors and spatio-temporal regulation of fusion?

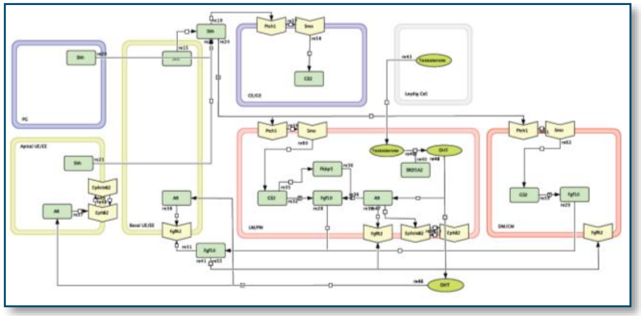
KEY EVENT: seam breakdown

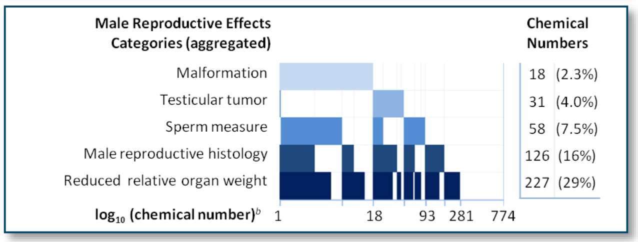
FLIPPING THE EGF/TGFβ3 SWITCH

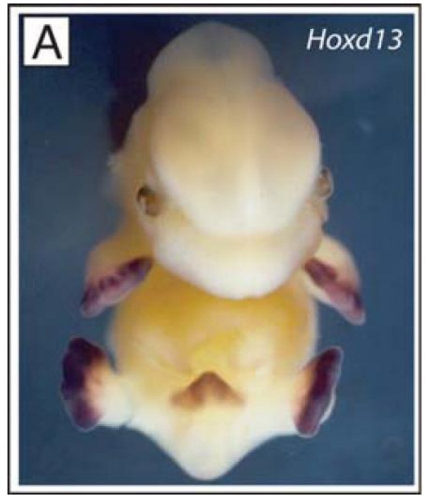


SOURCE: Hutson et al. (manuscript in preparation).

VIRTUAL GENITAL TUBERCLE







Data Mining

Modeling

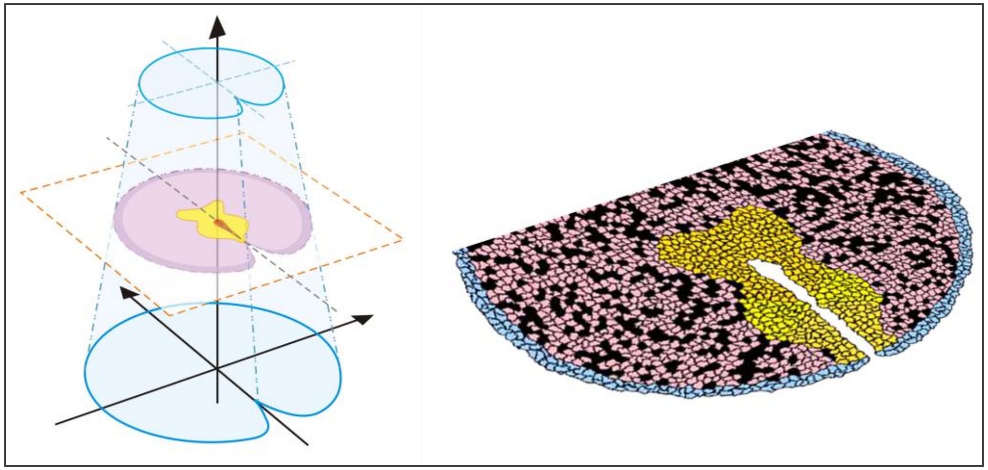
Urethral Closure Defects

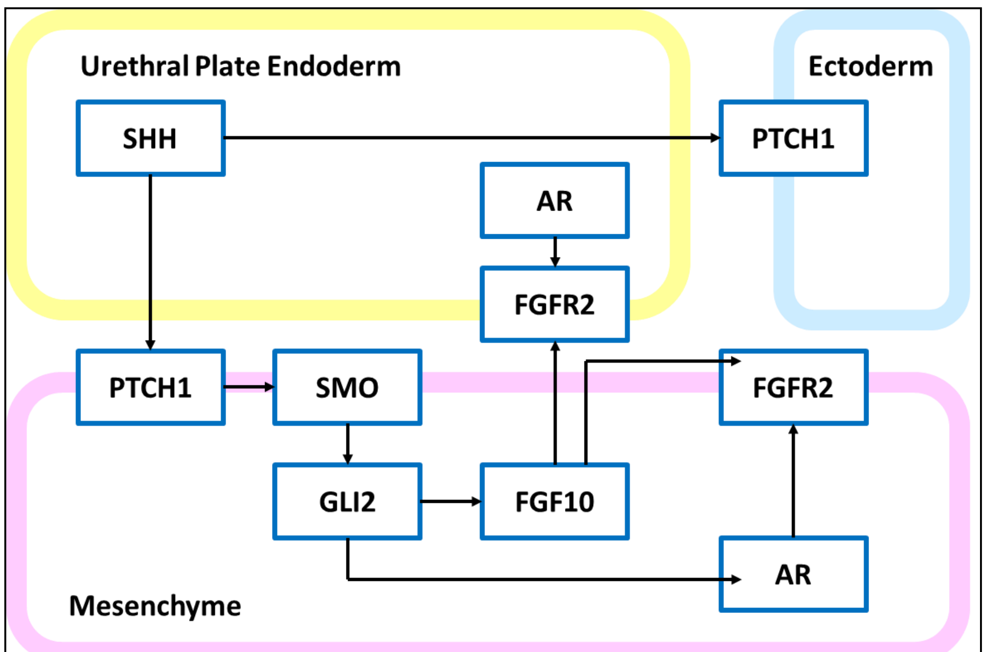
What pathways are relevant to TDS/hypospadias? (AOPs)

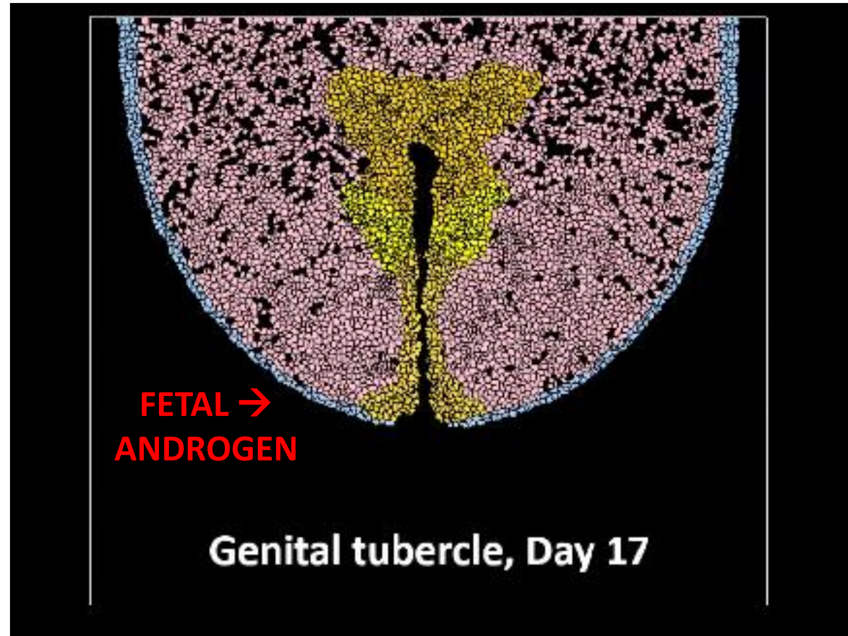
How can we model the underlying GT biology and cellular dynamics? (VTMs)

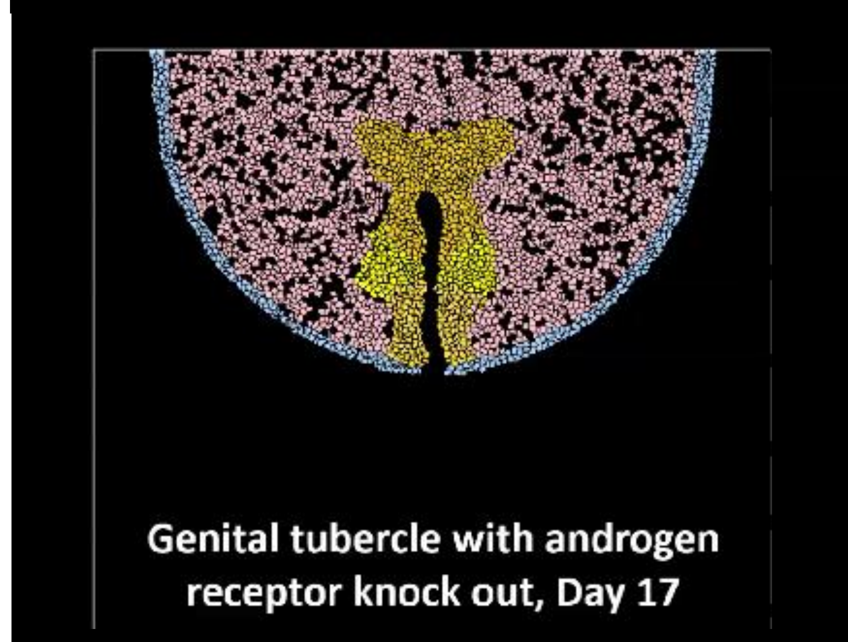
How do androgen-responsive pathways influence specific cellular behaviors and spatio-temporal regulation of GT development?

VIRTUAL GENITAL TUBERCLE (preliminary)









Some Benefits and Challenges For Predictive Toxicology:

- *in silico* reconstruction of multicellular systems to elevate HTS data
- 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)
- quantifying the 'un-measureable' (lesion propagation)
- 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)
- extending them for lifestage considerations / life-course models