# Application of AOPs to Developmental Outcomes

*case study on developmental vascular toxicity*

*DISCLAIMER: The views expressed are those of the presenter and do not necessarily reflect Agency policy.*

**Thomas B. Knudsen, PhD**
Developmental Systems Biologist
US EPA, National Center for Computational Toxicology
knudsen.thomas@epa.gov
ORCHID 0000-0002-5036-596x

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California

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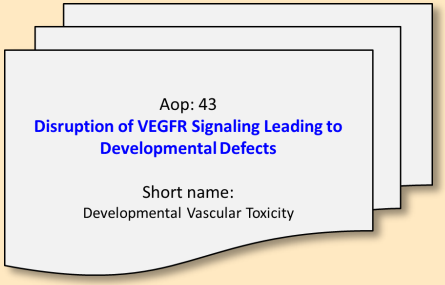
Teratology Society's 59th Annual Meeting

# Vascular Development

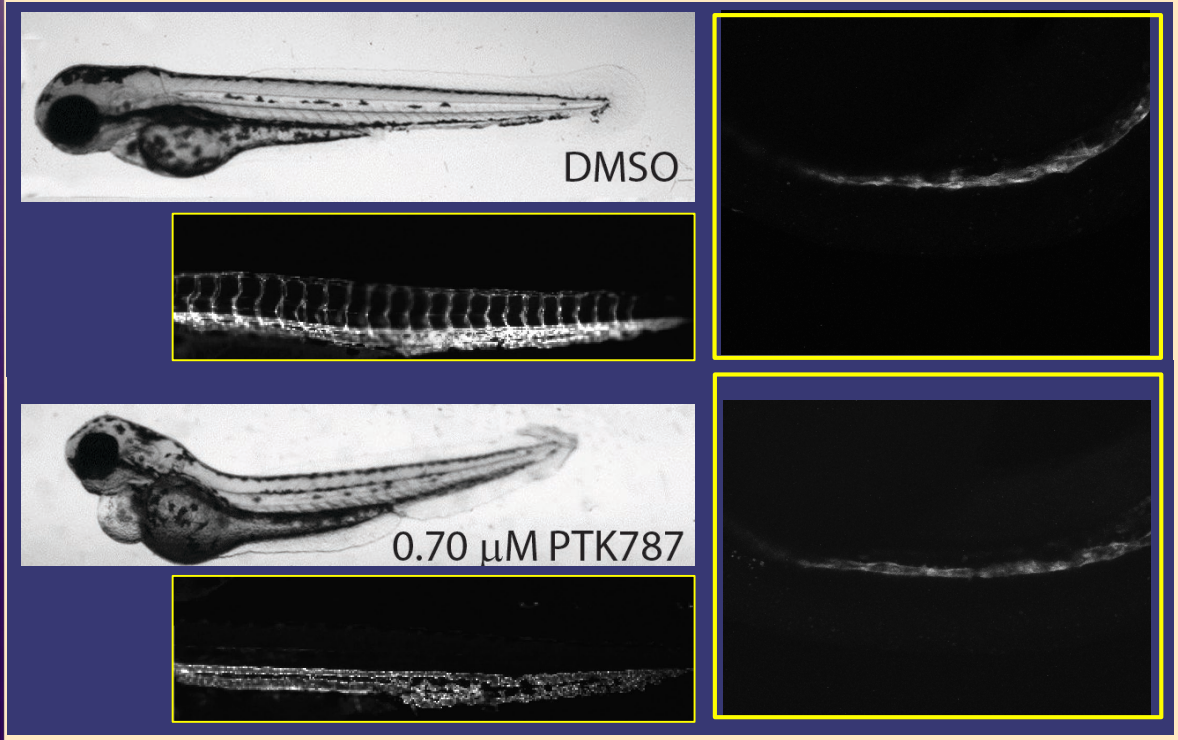
- Blood vessel formation is essential to embryogenesis (cardiovascular is first functioning organ system across *Vertebrate* species).

- Vascular insufficiency is tied to many disease processes (diabetes, preeclampsia, neonatal respiratory distress, osteoporosis, teratogenesis, …).

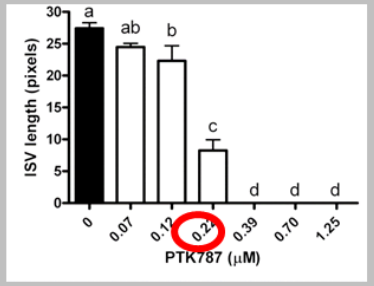
- Aop43: one of 28 AOPs included in the OECD work plan with status ‘open for citation & comment’ [https://aopwiki.org/wiki/index.php/Aop:43].



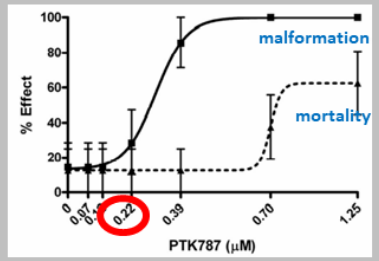

# VEGFR2 inhibition (PTK787)



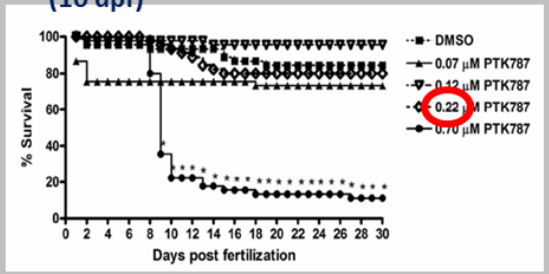

ISV length (72 hpf)




Terata (120 hpf)

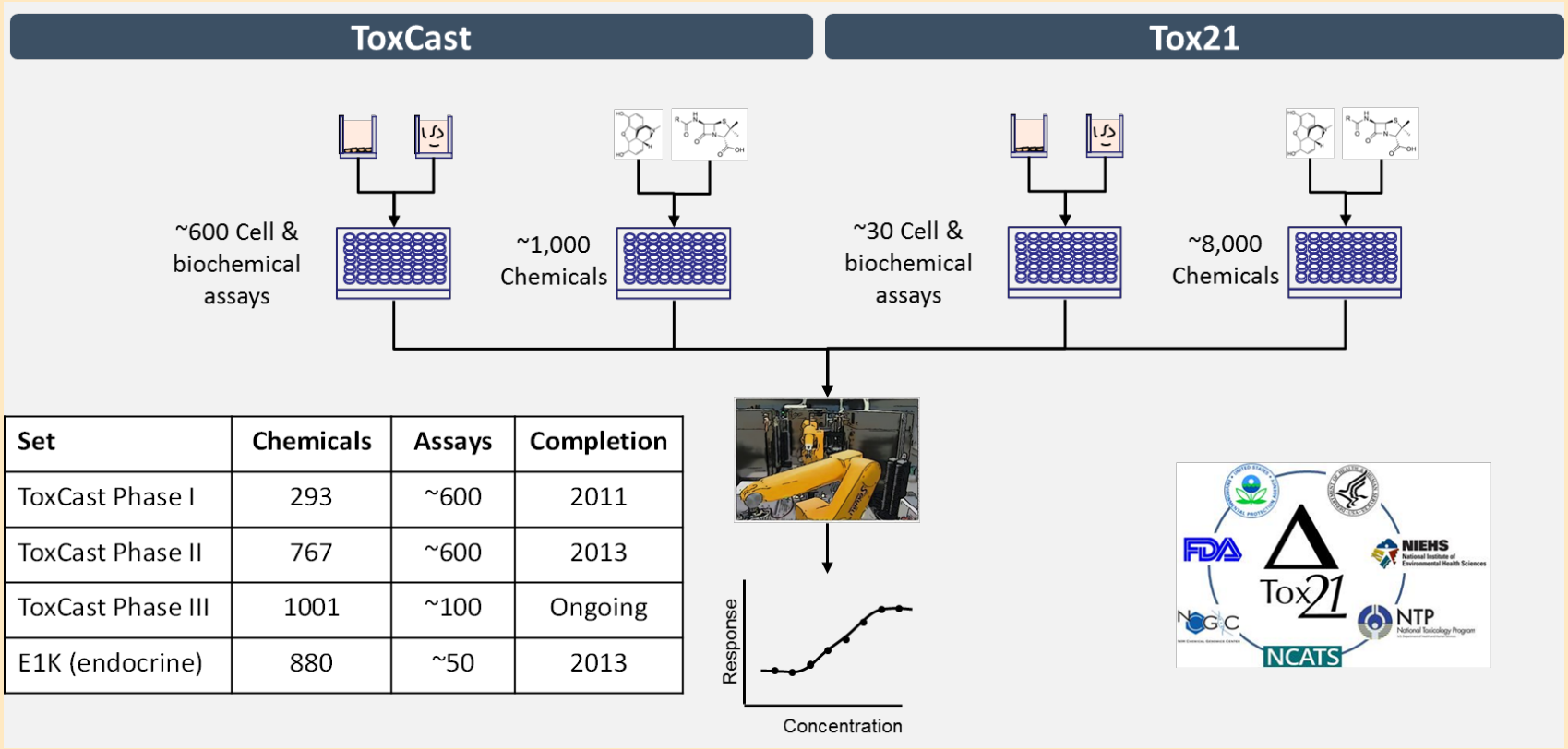



Lifespan (10 dpf)




*SOURCE: Tal et al. (2014) Reprod Toxicol*

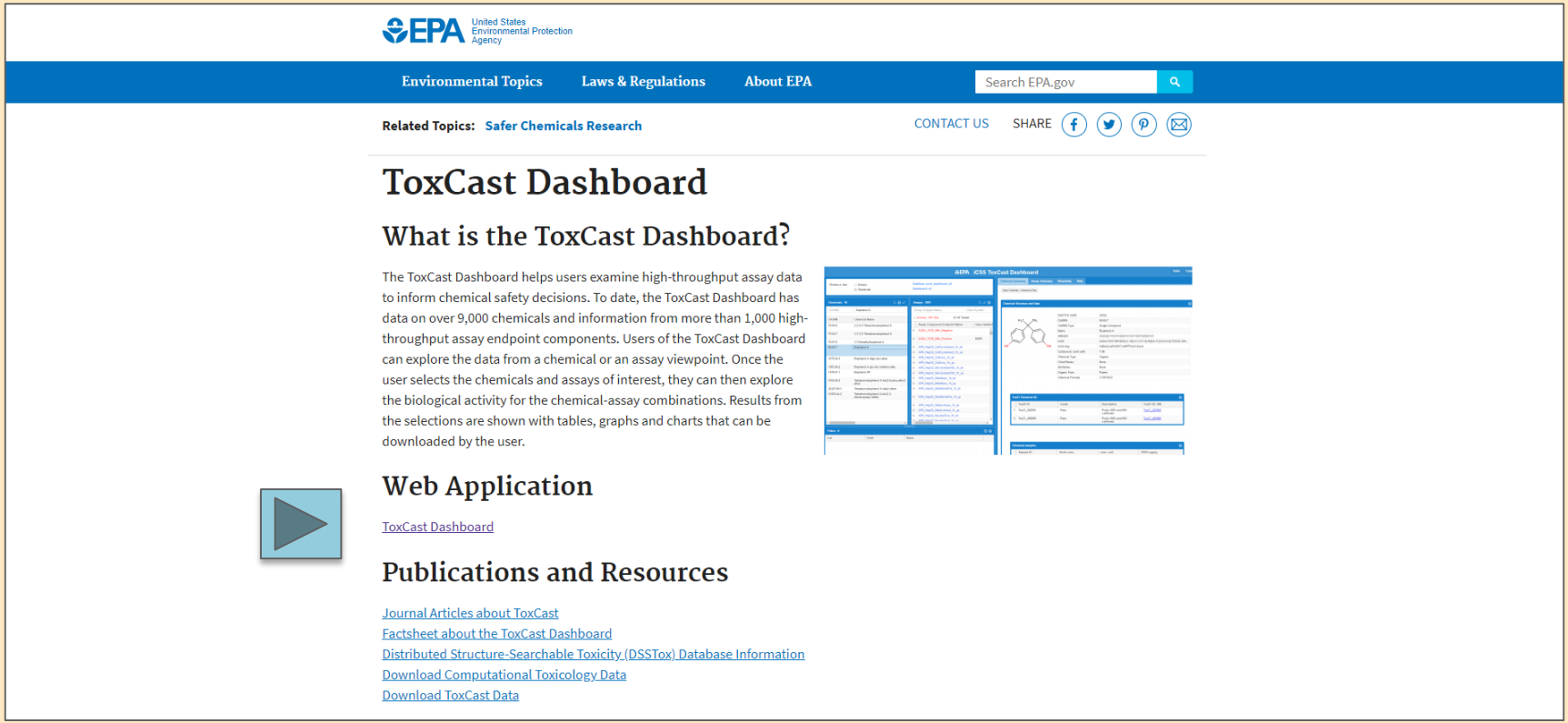
# Shifting toxicology to pathway-based approaches




| Set | Chemicals | Assays | Completion |
|---|---|---|---|
| ToxCast Phase I | 293 | ~600 | 2011 |
| ToxCast Phase II | 767 | ~600 | 2013 |
| ToxCast Phase III | 1001 | ~100 | Ongoing |
| E1K (endocrine) | 880 | ~50 | 2013 |

https://www.epa.gov/chemical-research/toxcast-dashboard

https://www.epa.gov/chemical-research/toxcast-dashboard



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# ToxCast Dashboard

## What is the ToxCast Dashboard?

The ToxCast Dashboard helps users examine high-throughput assay data to inform chemical safety decisions. To date, the ToxCast Dashboard has data on over 9,000 chemicals and information from more than 1,000 high-throughput assay endpoint components. Users of the ToxCast Dashboard can explore the data from a chemical or an assay viewpoint. Once the user selects the chemicals and assays of interest, they can then explore the biological activity for the chemical-assay combinations. Results from the selections are shown with tables, graphs and charts that can be downloaded by the user.

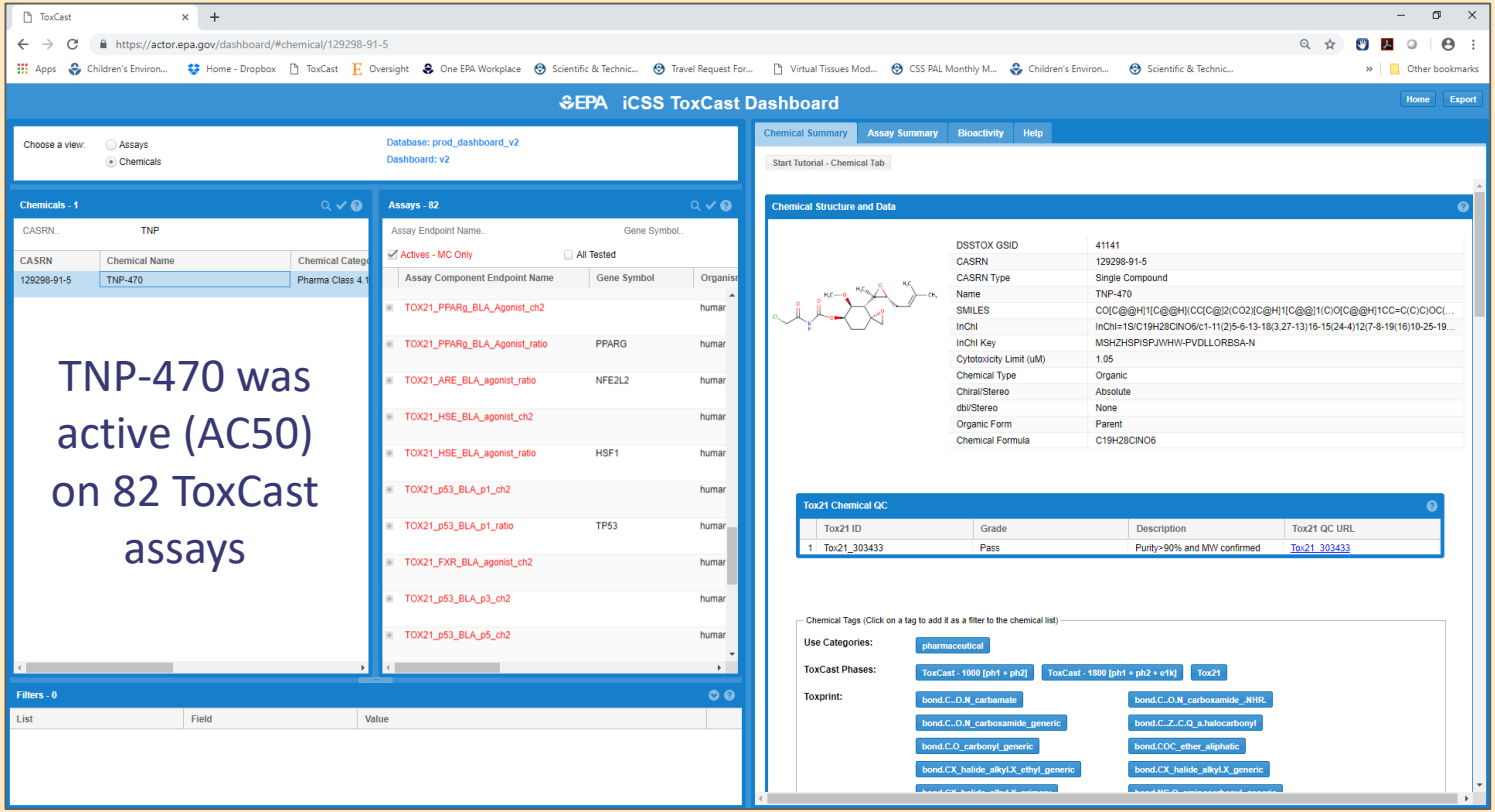
## Web Application

ToxCast Dashboard

## Publications and Resources

Journal Articles about ToxCast
Factsheet about the ToxCast Dashboard
Distributed Structure-Searchable Toxicity (DSSTox) Database Information
Download Computational Toxicology Data
Download ToxCast Data

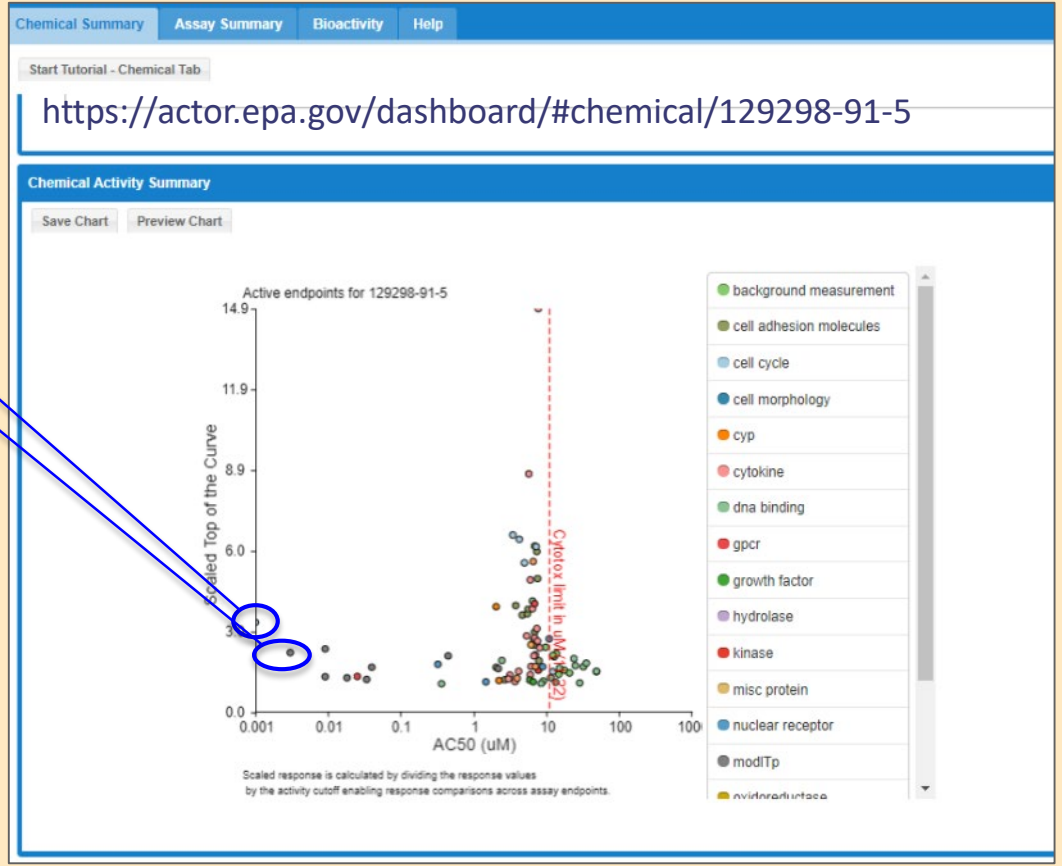
# **Example:** TNP-470, an anti-angiogenic pharma compound



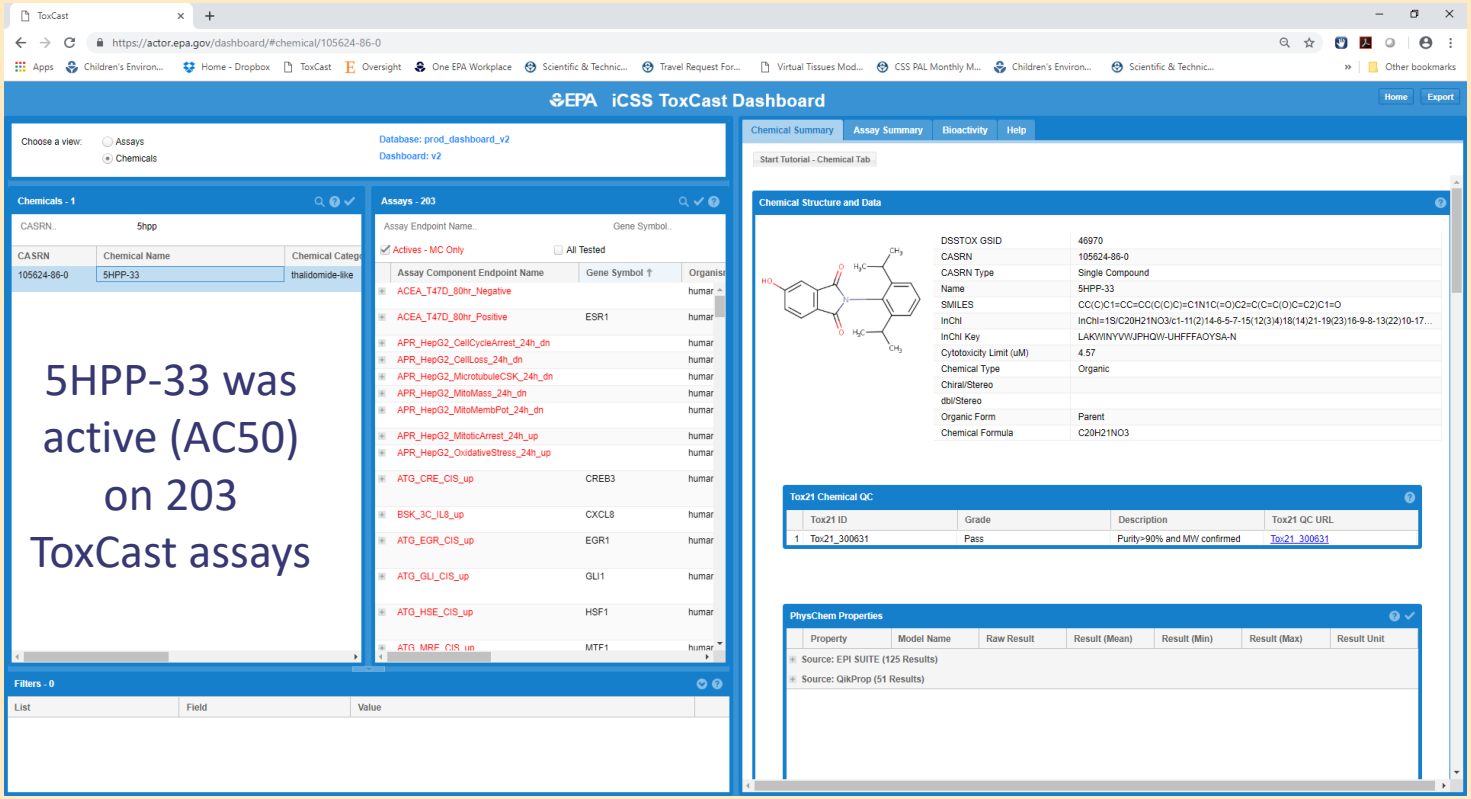

TNP-470 was active (AC50) on 82 ToxCast assays

https://actor.epa.gov/dashboard/

↓BSK_3C (coronary) proliferation
zebrafish yolk sac edema



# **Example:** 5HPP-33, an anti-angiogenic pharma compound

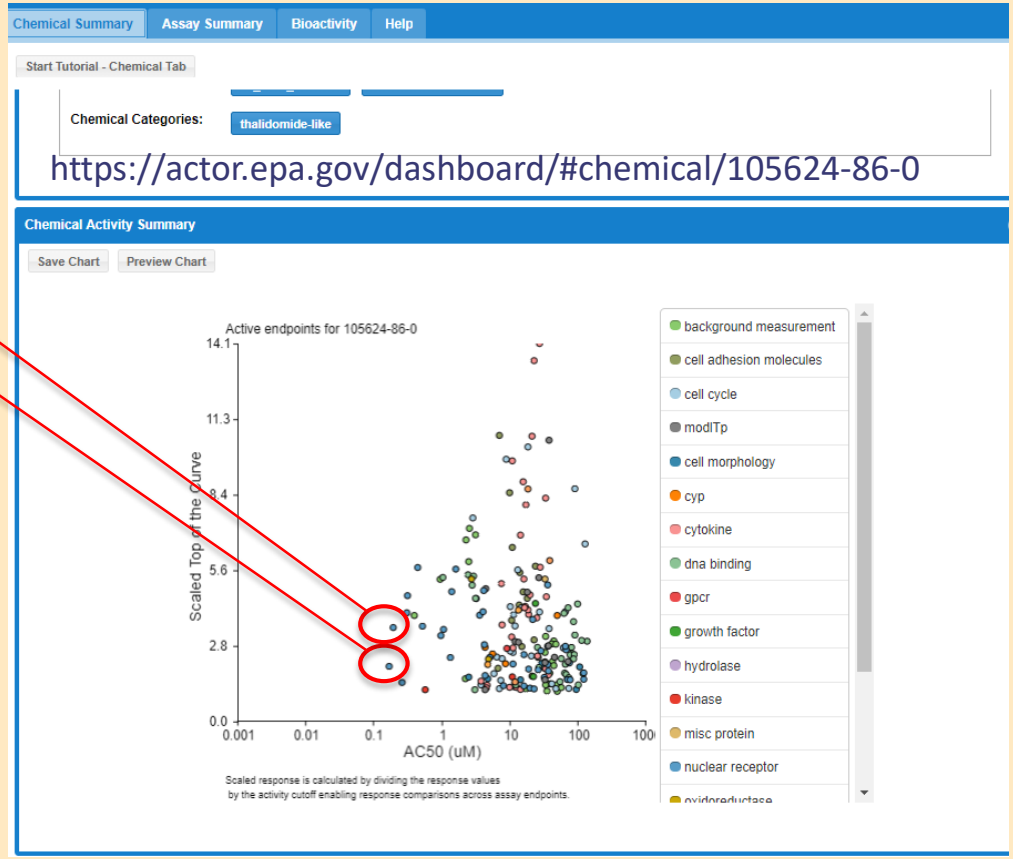



5HPP-33 was active (AC50) on 203 ToxCast assays

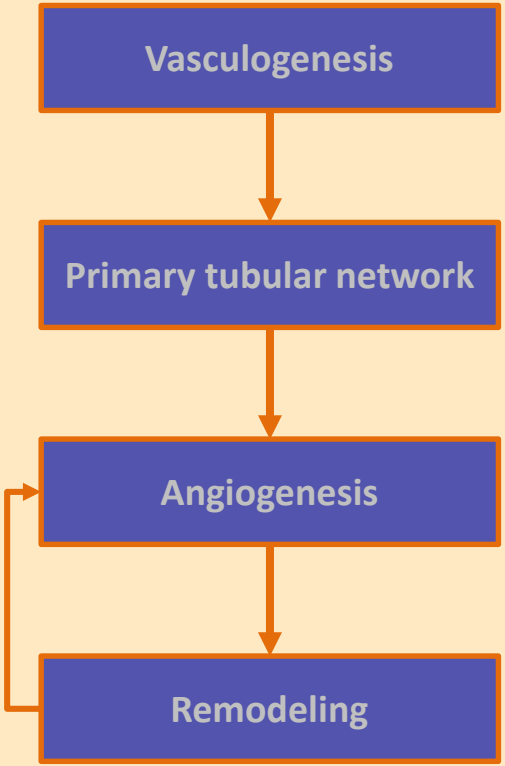
https://actor.epa.gov/dashboard/

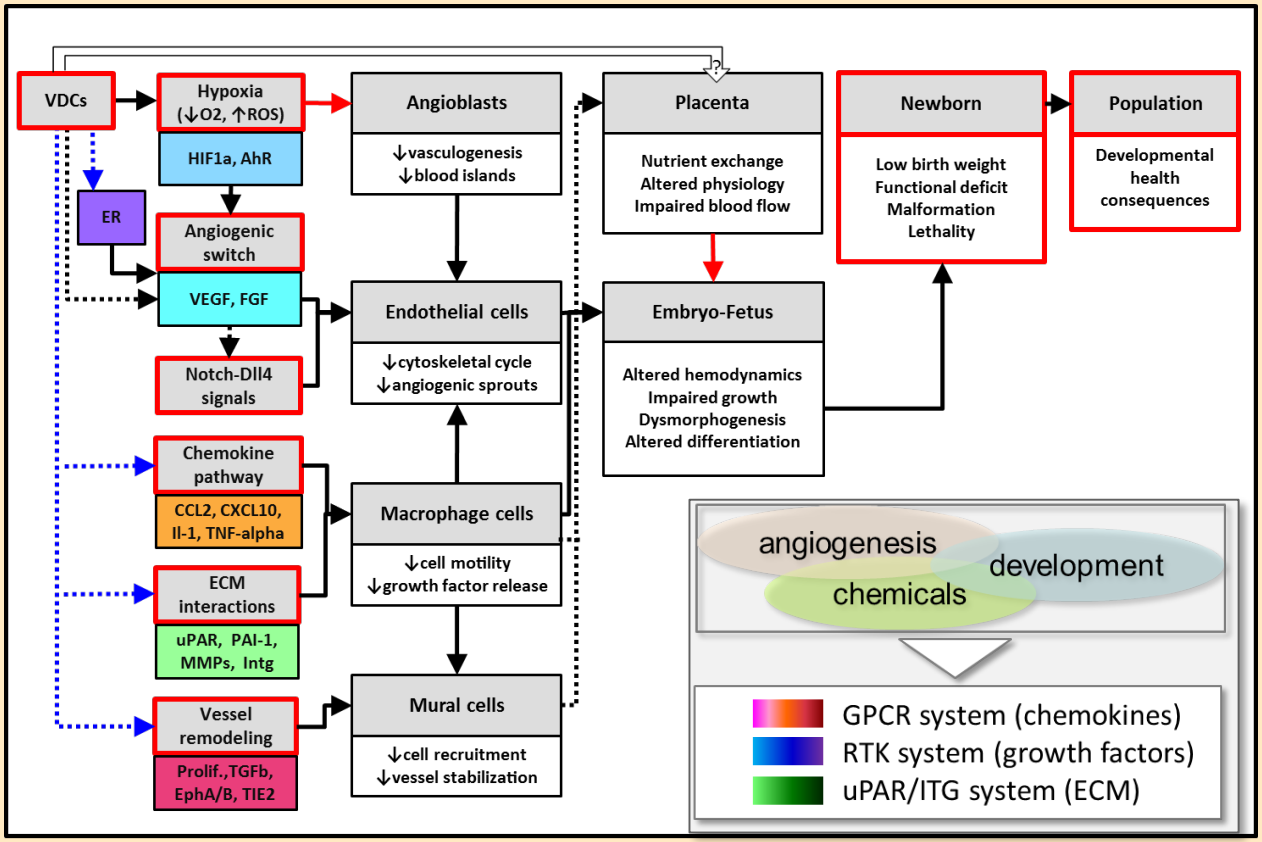
↑TOX21_ERa agonist

↑ATG_ERa transactivation



# AOP framework: developmental vascular toxicity (DVT)

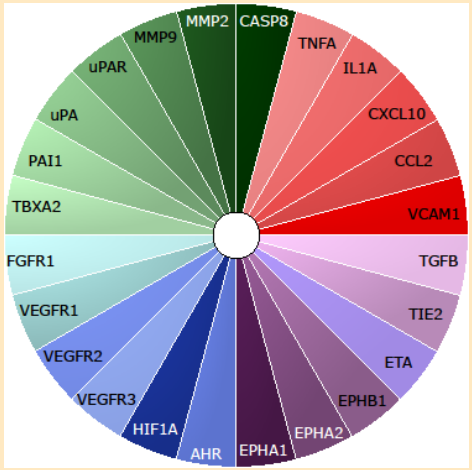


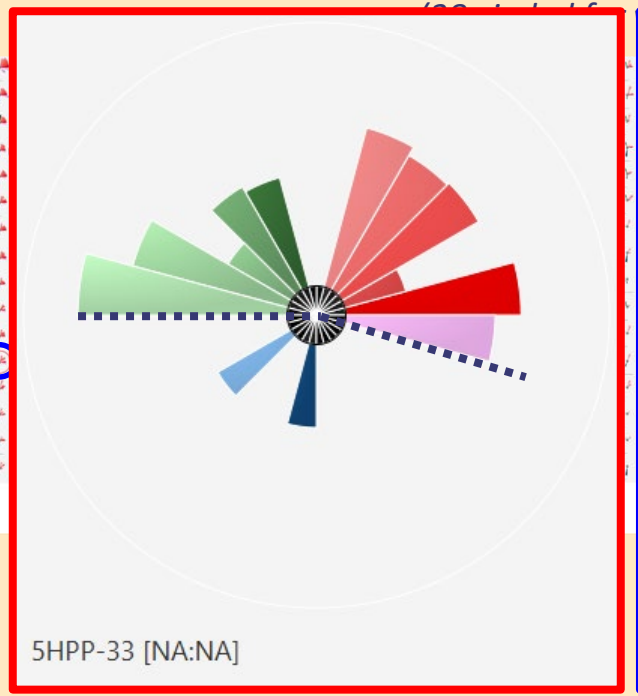

*SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res*

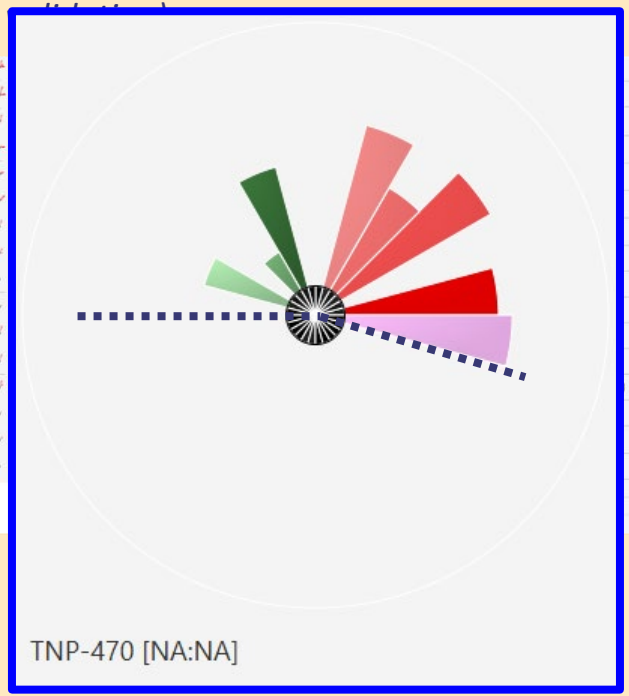
# **AOP-based ranking:** predicted vascular disrupting chemicals (pVDCs)

*24 ToxCast target assays (pVDC ToxPi)*

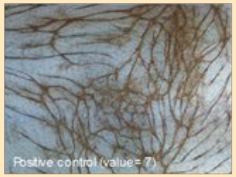


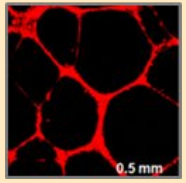

*1058 ToxCast chemicals ranked by pVDC ToxPi*

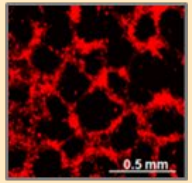


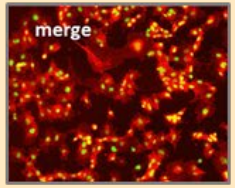



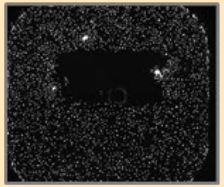

*SOURCE: Saili et al. (submitted)*

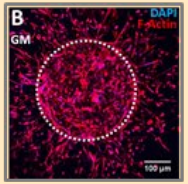


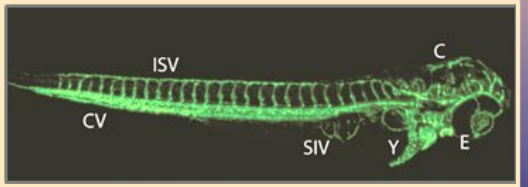



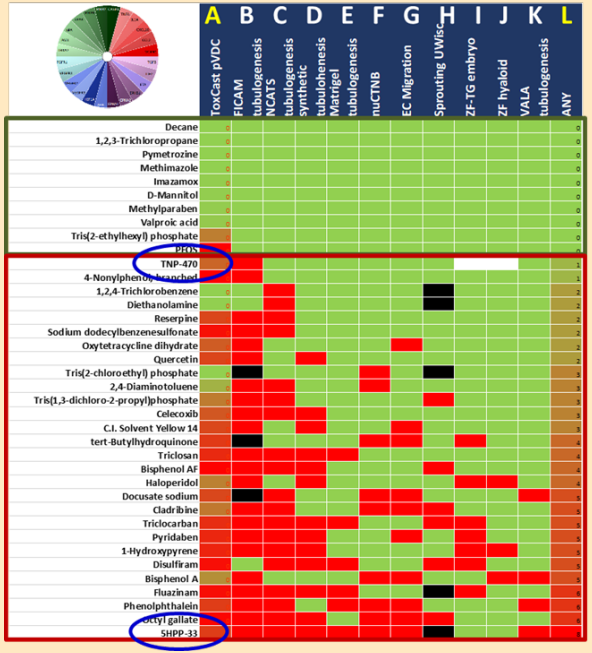






- inactive
- active
- cytotoxic
- no data

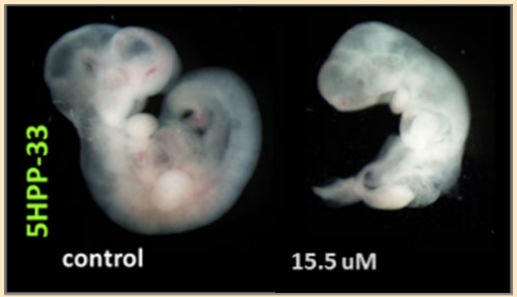


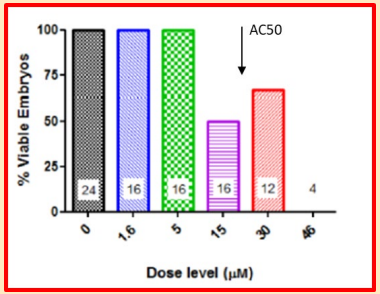

A pVDC ToxPi
B HUVEC tubulogenesis (FICAM)
C HUVEC tubulogenesis (NCATS)
D tubulogenesis in synthetic matrices (HMAPS)
E tubulogenesis in Matrigel (HMAPS)
F nuCTNB biomarker (VALA)
G endothelial cell migration (VALA)
H iPSC endothelial sprouting (HMAPS)
I ISV reporter zebrafish (NHEERL)
J reporter zebrafish (UDUBLIN)
K HUVEC tubulogenesis (VALA)
L ANY (B to K)

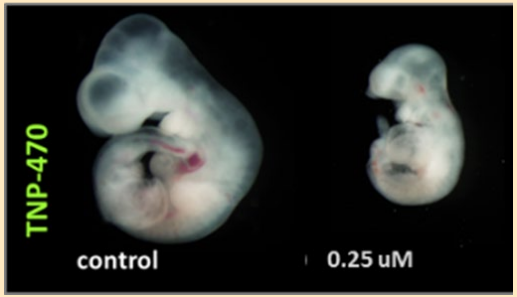
**sensitivity 0.89, specificity 0.80**
**balanced accuracy 87% (PPV 93%, NPV 73%)**

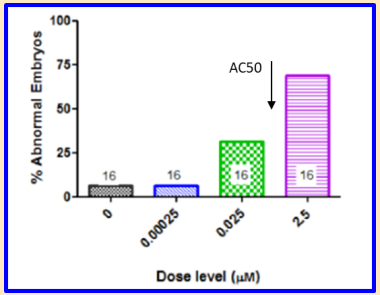
*SOURCE: Saili et al. (submitted)*

# **Embryotoxicity:** rat whole embryo culture




## 5HPP-33

- synthetic thalidomide analog
- microtubule disruptor
- critical effect ↓embryo viability
- AC50 = 21.2 µM

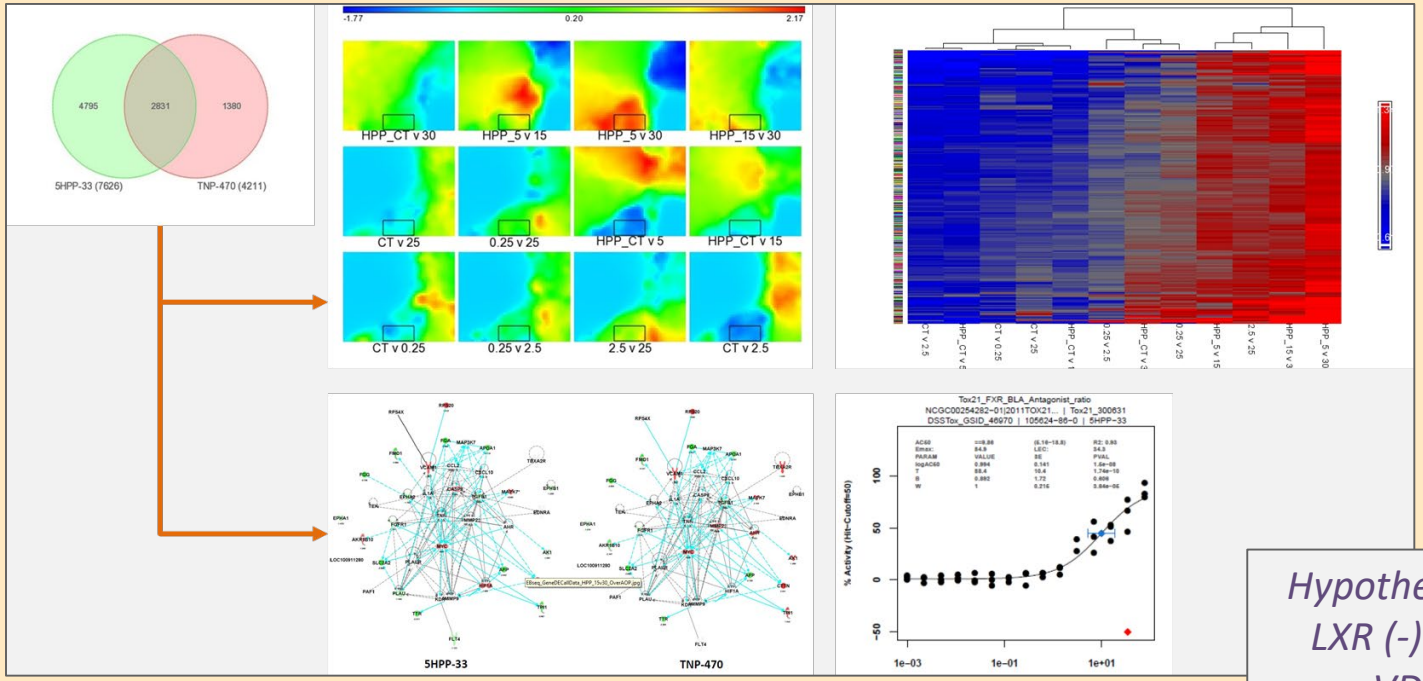
## TNP-470

- synthetic fumagillan analog
- MetAP II inhibitor
- critical effect ↑dysmorphogenesis
- AC50 = 0.038 µM

*SOURCE: Ellis-Hutchings et al. (2017) Reprod Toxicol*

# **RNAseq profile:** 5HPP-33 vs TNP-470 (4 hr whole embryo culture)

**2831 DEGs overlap** → **SOM (464 genes in ROI box)** → **ROI clusters**




- splicesome and RNA metabolism
- protesosome and ubiquitination

*Hypothesis: FXR (+) and LXR (-) may be KEs in pVDT pathways.*

*SOURCE: Saili et al. (submitted)*

# **Computer simulation:** cell agent based models (cABMs)

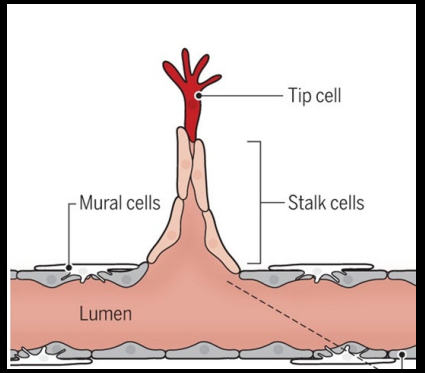
**Approach:** build and test self-organizing morphogenetic systems in silico using an open-source modeling environment (CompuCell3d.org).

**Input:** A.I. cast into mathematically-defined cells (agents), synthetic gene circuits, and viscoelastic properties to emulate developmental progression (embryogeny).

**Emergence:** simulation resolves into normal or perturbed phenotypes reading in vitro data input from specific ToxCast assays (cybermorphs).

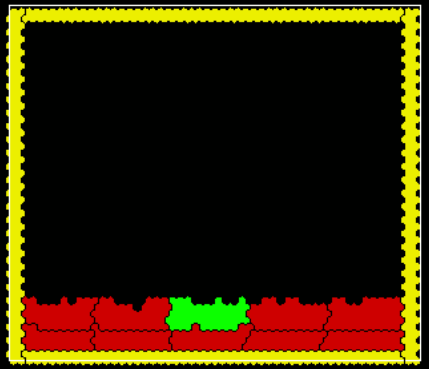
**Output:** probabilistic rendering of where, when and how a developmental defect might occur (critical phenomena).

# Cell Agent-Based Models (cABMs)




*Li and Carmeliet (2018) Science*

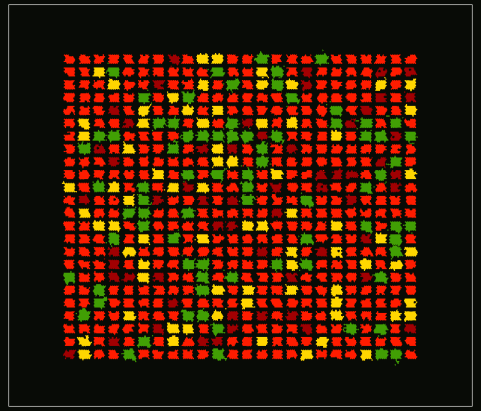
VEGF corridors



*Nicole Kleinstreuer*

***VEGF165***
***MMPs***
***VEGF121***
***sFlit1***
***TIE2***
***CXCL10***
***CCL2***

Network assembly

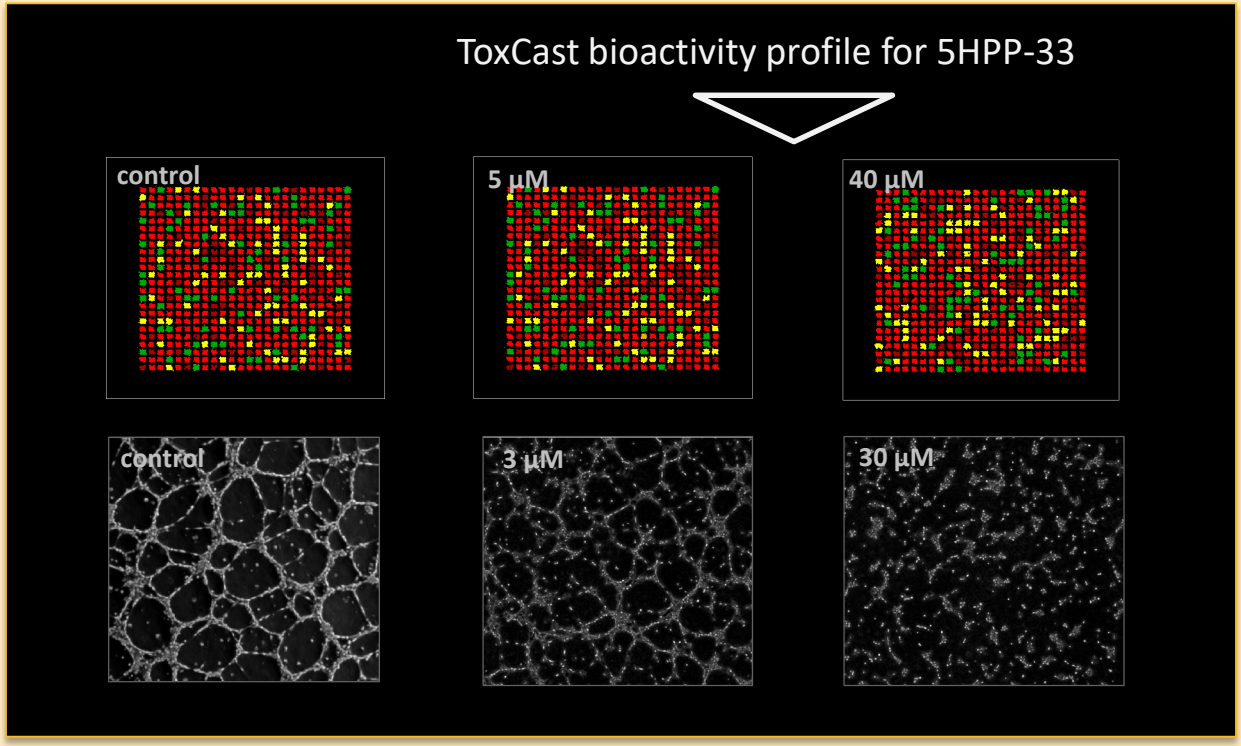


*Kleinstreuer et al. (2013) PLoS Comp Biol*

***Endothelial Stalk***
***Endothelial Tip***
***Mural Cell***
***Inflammatory Cell***

**SOFTWARE: [www.CompuCell3D.org](http://www.CompuCell3D.org)**
BioComplexity Institute, Indiana U

# Angiogenesis disruption: *in silico* simulation of the pVDC ToxPi

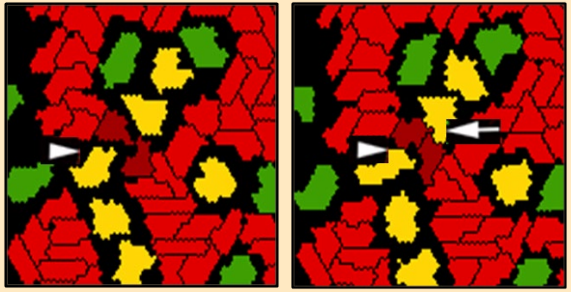



*SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol 9(4): e1002996*

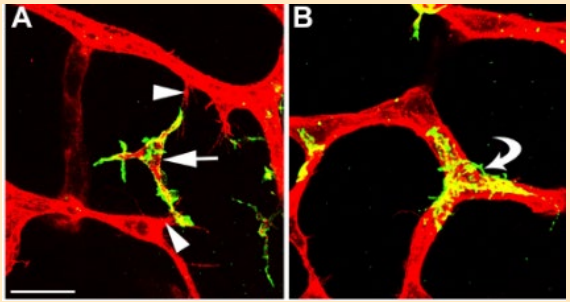
# **Smart Models:** emergence and self-organization

Time→

Kleinstreuer et al. (2013)



Fentin et al. (2010)




- *in silico* model spontaneously displayed co-opting of tip cells (dark red) by inflammatory cells (yellow) during endothelial network formation.
- recapitulates the natural phenomenon referred to as ‘macrophage bridging’ co-opting of during endothelial network formation.

Placeholder for any references / rights for images

## Summary: *“decoding the toxicological blueprint of vascular development”*

- AOP-based ontologies provide the necessary structure for quantitative prediction of cellular and tissue responses to molecular perturbation(s).
- For DevTox, this can be demonstrated by an AOP framework for embryonic vascular disruption represented in the OECD AOP-KB (Aop43).
- Because the ‘angiogenic cycle’ is responsive to diverse genetic and physiological signals, Aop43 provides a kernel for co-opting larger AOP networks.
- Computer modeling and simulation puts all key events into motion enabling a new way to predictively model multicellular complexity in a self-organizing ‘virtual’ system.