Adverse Outcome Pathway (AOP) Framework for Embryonic Vascular Disruption and Developmental Defects

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SOT symposium: Cardiopulmonary Consequences of Gestational Toxicant Exposure: Getting to the Heart of the Matter

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DISCLAIMER: The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the US EPA.

DISCLOSURES

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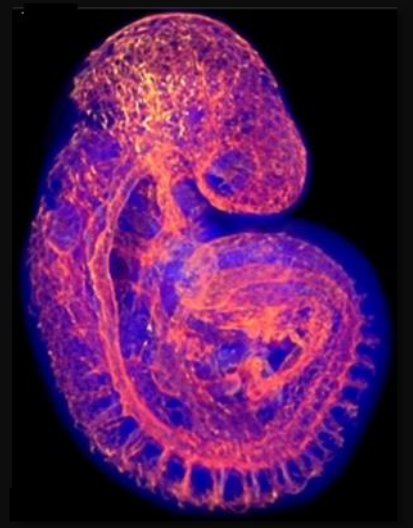
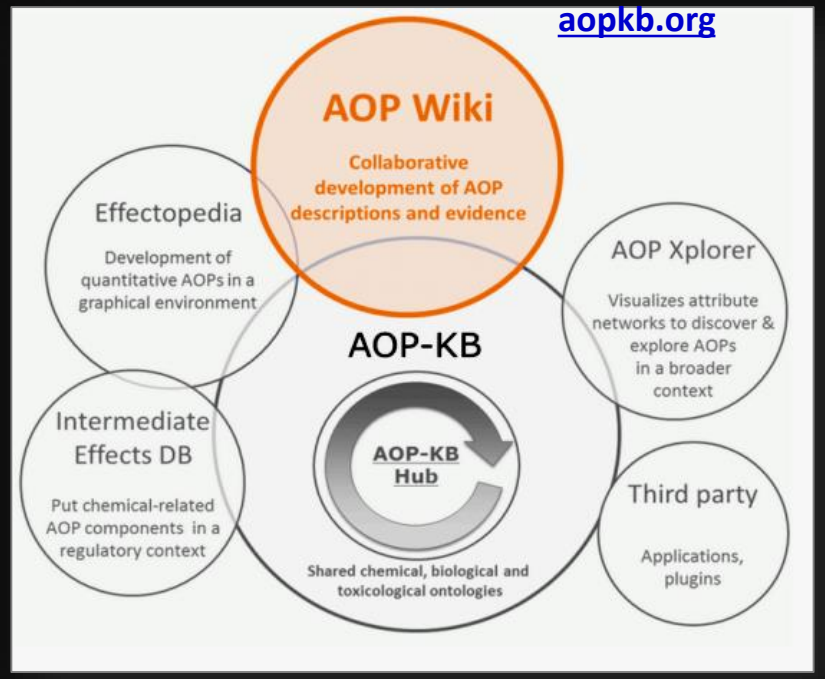
Vascular Disruption and Developmental Toxicity



- *In utero* vascular disruptions are associated with a variety of birth defects
  Husain et al. 2008, Birth Defects Res A
- Vascular disruption is one of 6 teratogenic mechanisms linked with meds taken by WOCBP
  van Gelder et al. 2010, Human Repro Update 16
- Vascular defects are the most common apparent cause of limb deficiencies in humans
  Gold et al. 2011, Am J Med Gen A
- Susceptibility to Thalidomide linked to the disruption of immature angiogenic network
  Therapontos et al. 2009, PNAS 106
- Predicted vascular disrupting chemicals in ToxCast correlate with developmental toxicity
  Kleinstreuer et al. 2011, Environ Hlth Persp 119
- Many genetic and environmental factors alter molecular pathways regulating angiogenesis
  Knudsen and Kleinstreuer 2011, Birth Defects Res C 93

Adverse Outcome Pathways

- HTS assays harness data on many chemicals but predictive toxicology's challenge is using the new data streams for hazard identification and regulatory decisions.
- Considerable mechanistic data exists in the literature (QSAR and Read-Across, 'omics, HCI, SMOs) but is under-utilized for regulatory purposes.
- An AOP points to a biological perturbation → specific adverse outcome and measures how we think it happens.
- AOP knowledgebase: compendium of AOPs with relevance to mode-of-action for specific chemicals.



SOURCE: Dan Villeneuve, USEPA

AOPs with OECD Status

TFHA/WNT Endorsed
Open for citation & comment = 6 AOPs

EAGMST Approved
Open for citation & comment = 1 AOP

EAGMST Under Review
Open for citation & comment = 10 AOPs
Open for comment. Do not cite = 8 AOPs

EAGMST Under Review
Open for citation & comment = 10 AOPs
Open for comment. Do not cite = 8 AOPs

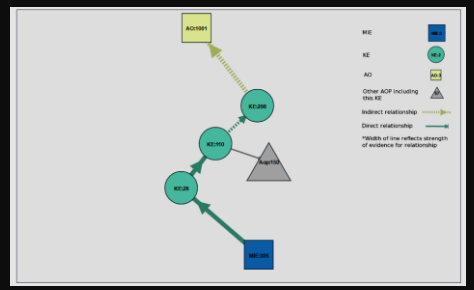
Under Development
Not open for comment = ~50 AOPs

Aop: 43

Disruption of VEGFR Signaling Leading to Developmental Defects

Short name:
Developmental Vascular Toxicity

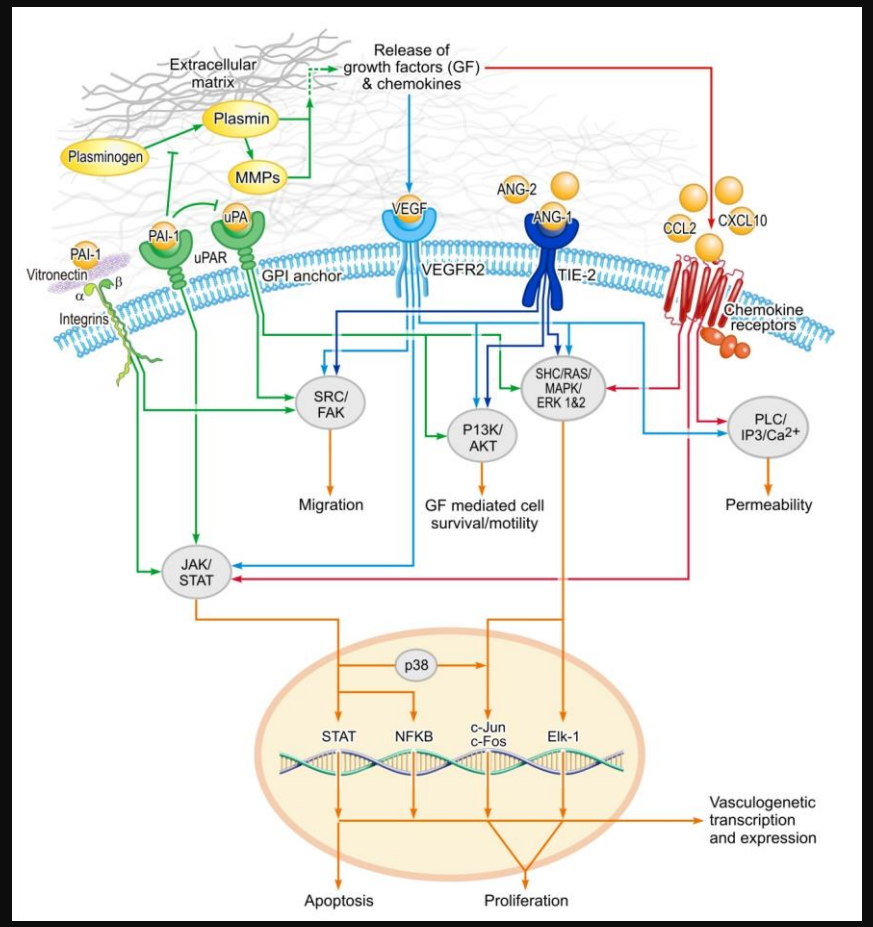
https://aopwiki.org/aops/43

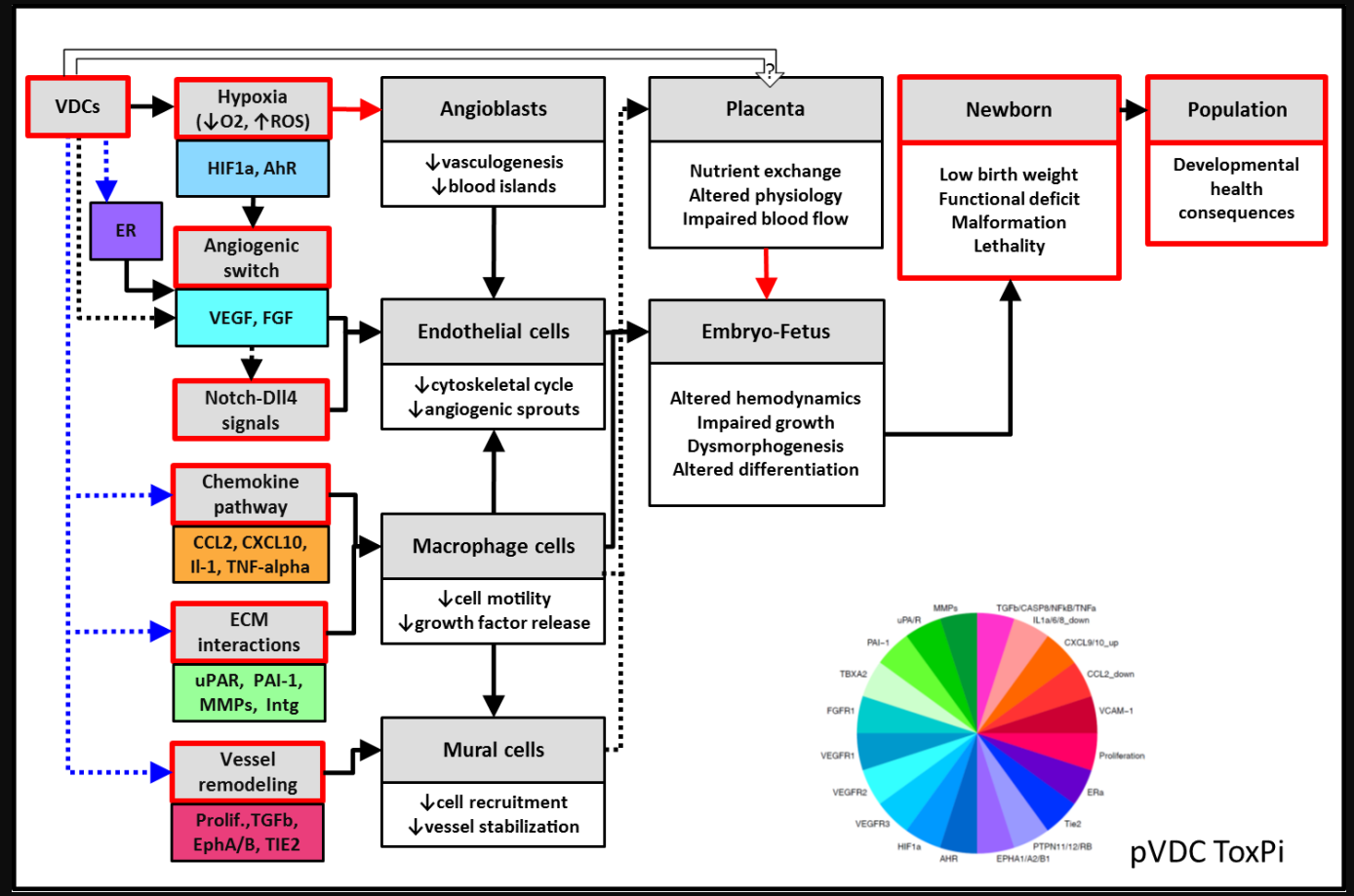


Aop43: Disruption of VEGF Signaling Leading to Developmental Defects

- Activation of VEGFR2 triggers angiogenic sprouting during early development and flow-sensing angio-adaptation later in development.
- **MIE:** molecular initiating event, VEGFR2 inhibition, may be invoked via ↓ VEGFA (production, mobility, liganding) or ↓ VEGFR2 (density, function, transduction).
- **KEs:** downstream key events on endothelial tip cells (exploratory behavior, cell migration) and endothelial stalk cells (cell proliferation, apoptosis).
- **KERs:** KE relationships with other cell interactions (stromal cells, macrophages), extracellular matrix (ECM) and micro-physiology (hemodynamics, metabolism).
- **AO:** adverse outcomes vary by anatomical region, organ system, gestational stage and state of the embryo, fetus or placenta.

AOP FRAMEWORK

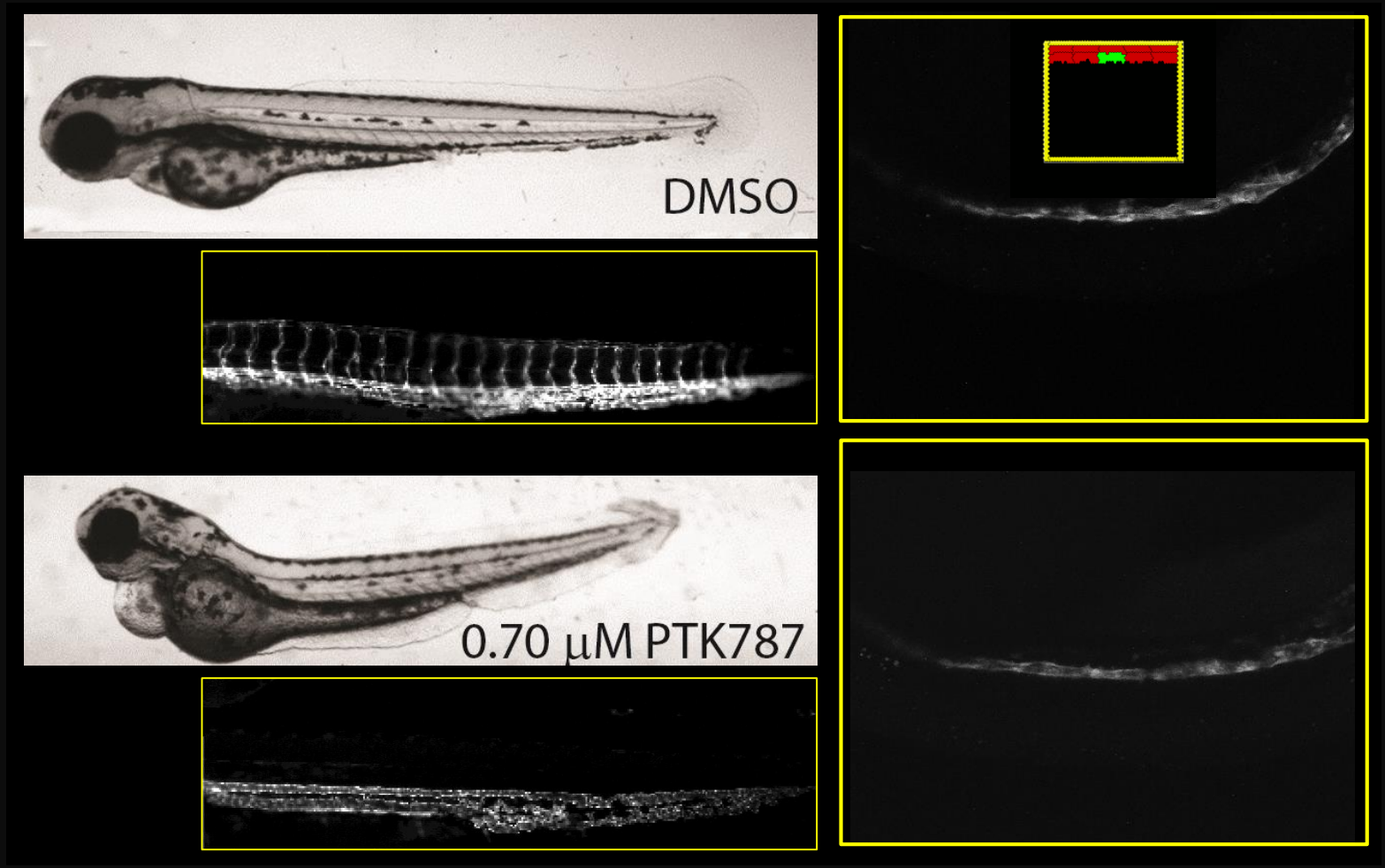




SOURCE: Kleinstreuer et al. 2011, Env Hlth Persp 119

SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res. C 93

VEGFR2 Inhibition (PTK787) and Angiodysplasia



SOURCE: Tal et al. 2014, Reprod Toxicol 48

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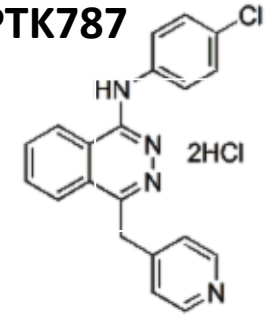
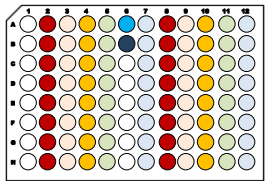
Hypothesis: disruption of angiogenesis is a key event in zebrafish developmental toxicity.

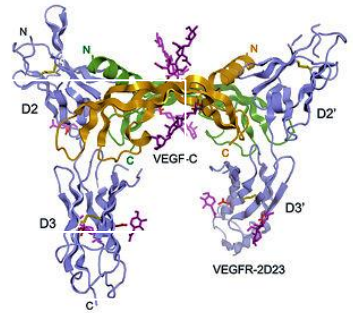
PTK787



exposure window

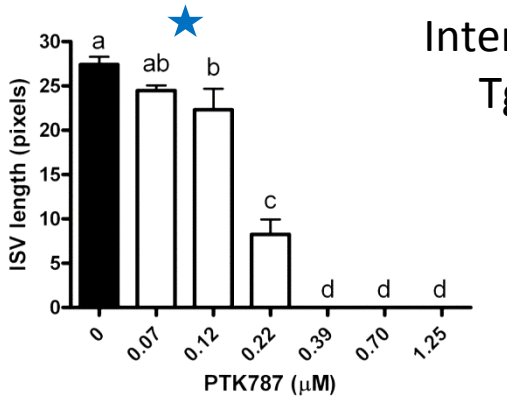
24-72 hpf

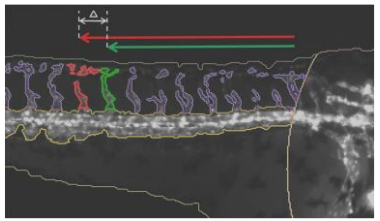




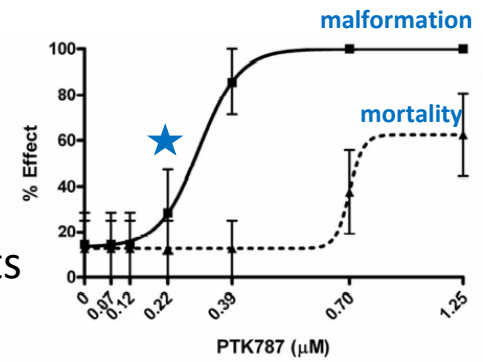
MIE: VEGFR2

InterSomitic Vessel length in Tg(kdrl:EGFP) embryos (0.07 uM at 72 hpf)

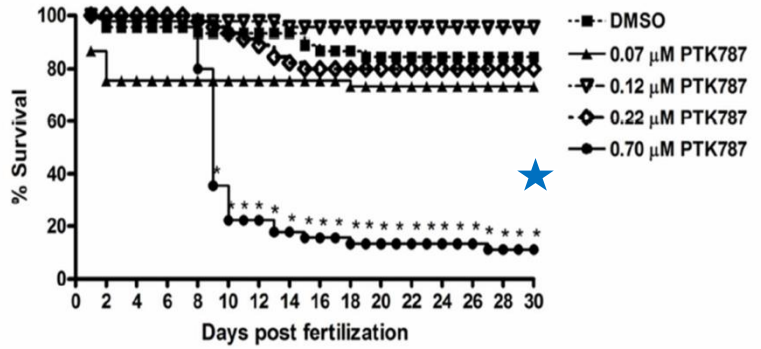




Developmental Effects (0.22 uM at 120 hpf)

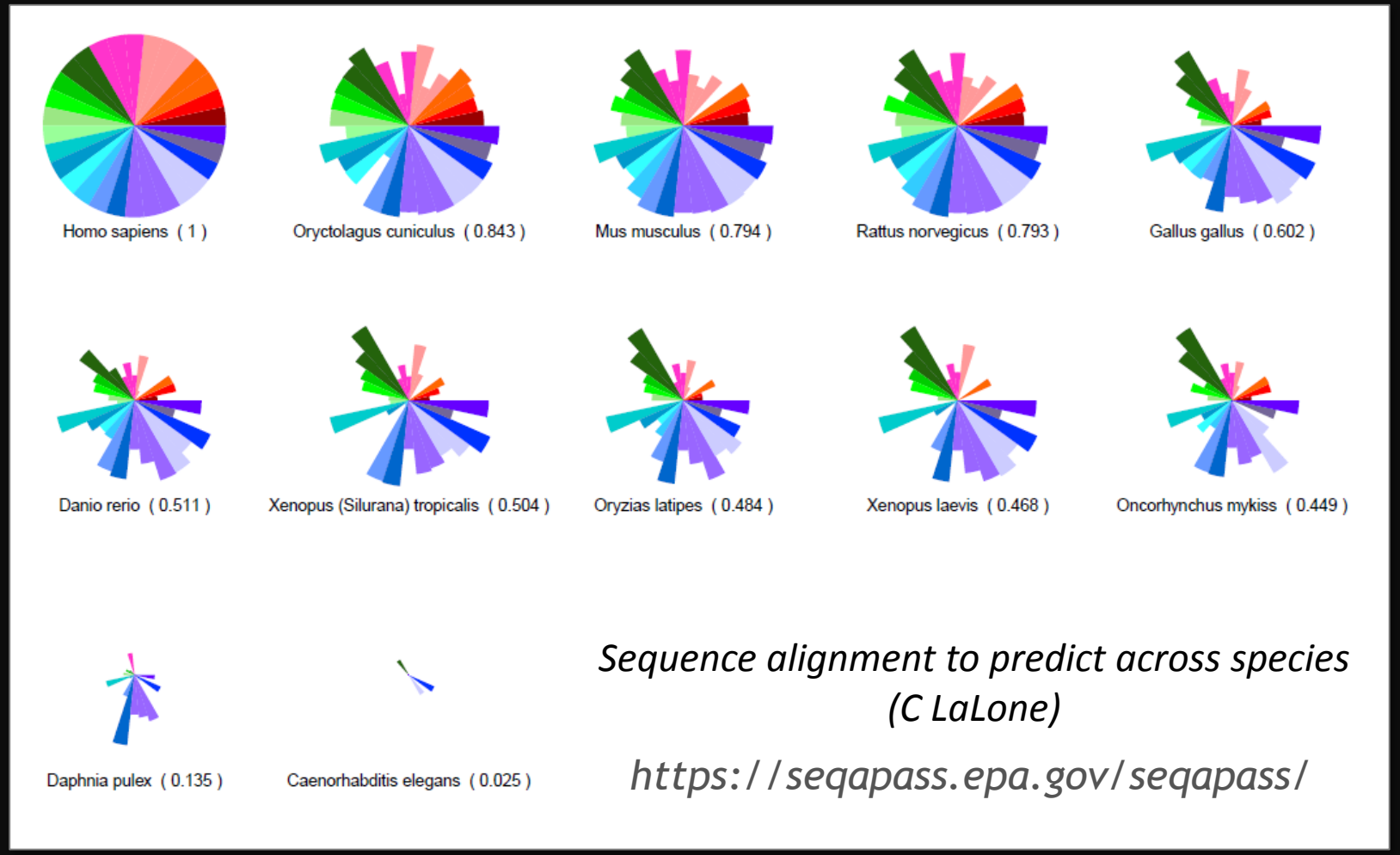


Loss of survival (0.70 uM by 10 dpf)



SOURCE: Tal et al. (2014), Reprod Toxicol 48

Taxonomic applicability: pVDC ToxPi protein sequence alignment

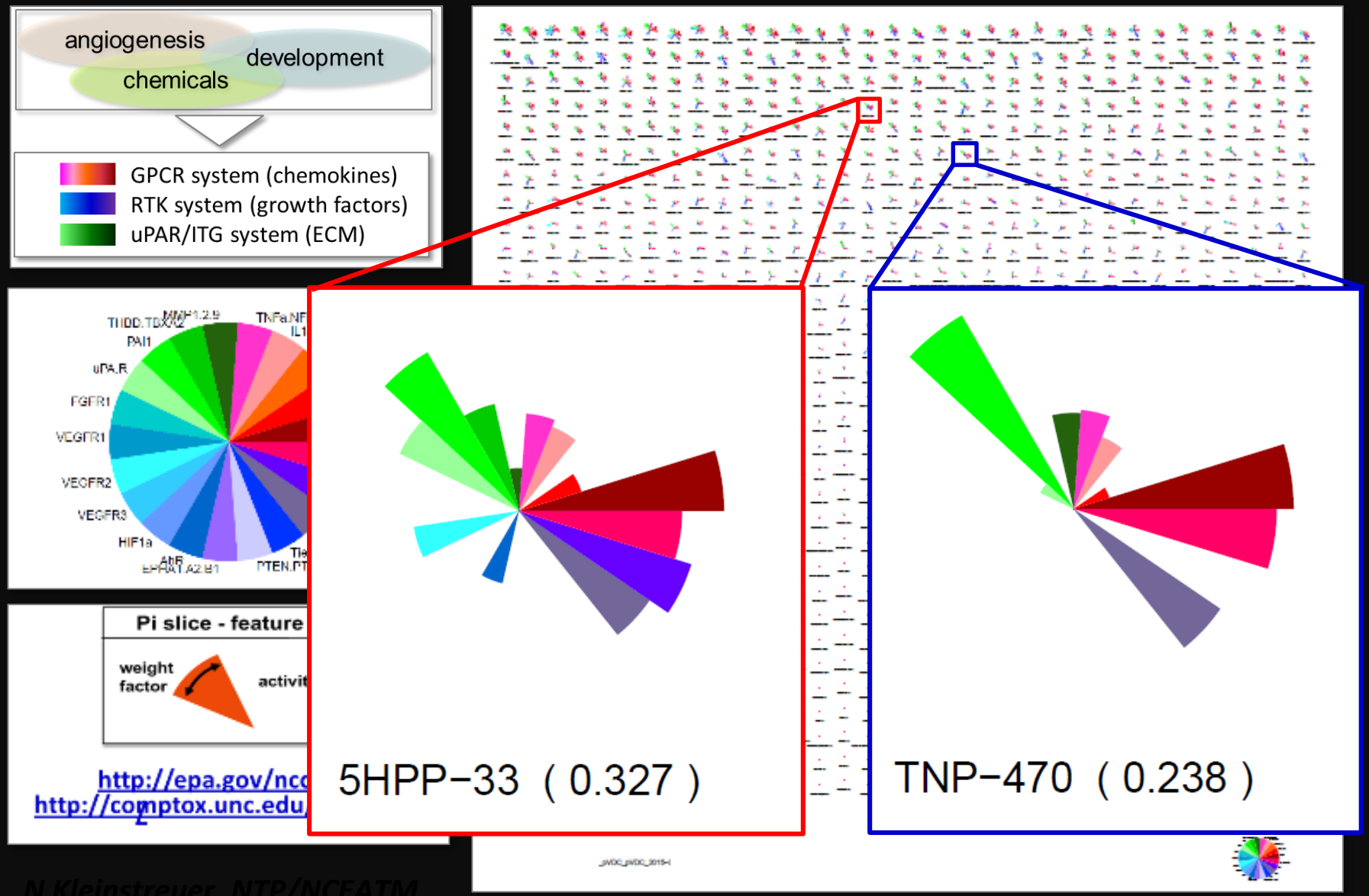


Sequence alignment to predict across species (C LaLone)

https://seqapass.epa.gov/seqapass/

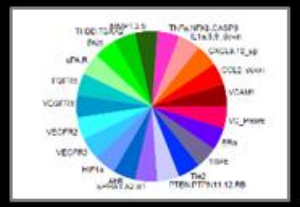
SOURCE: Tal et al. 2017, Reprod Toxicol (in press)

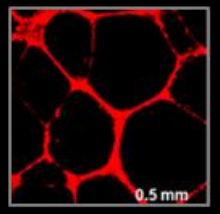
Angiogenesis: chemicals sorted by predicted potential to disrupt angiogenesis (pVDCs)

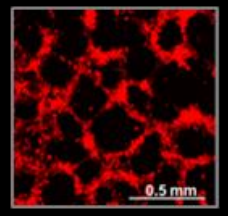


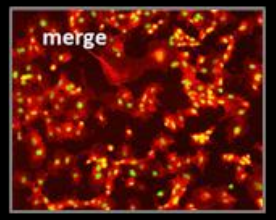
N Kleinstreuer, NTP/NCEATM

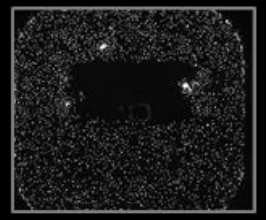
Subset (36 + 2): qualification of pVDC ToxPi across 8 platforms

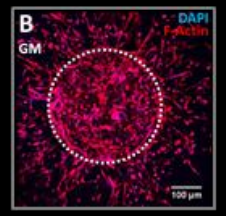


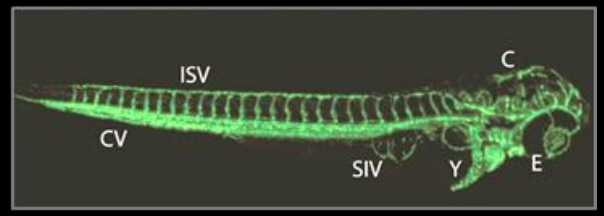


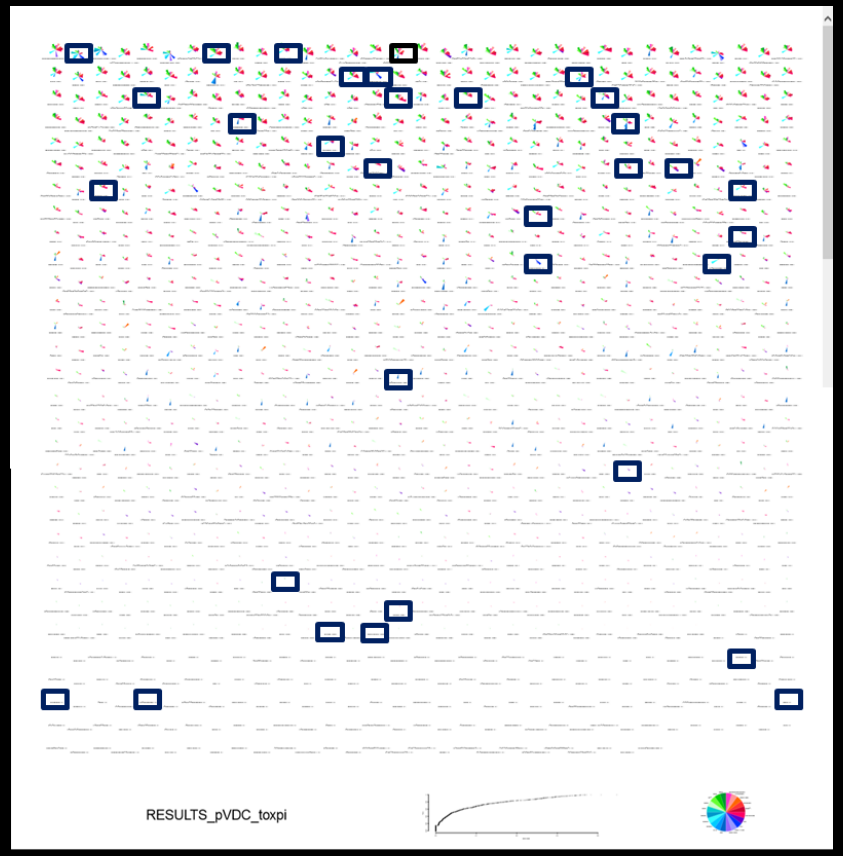


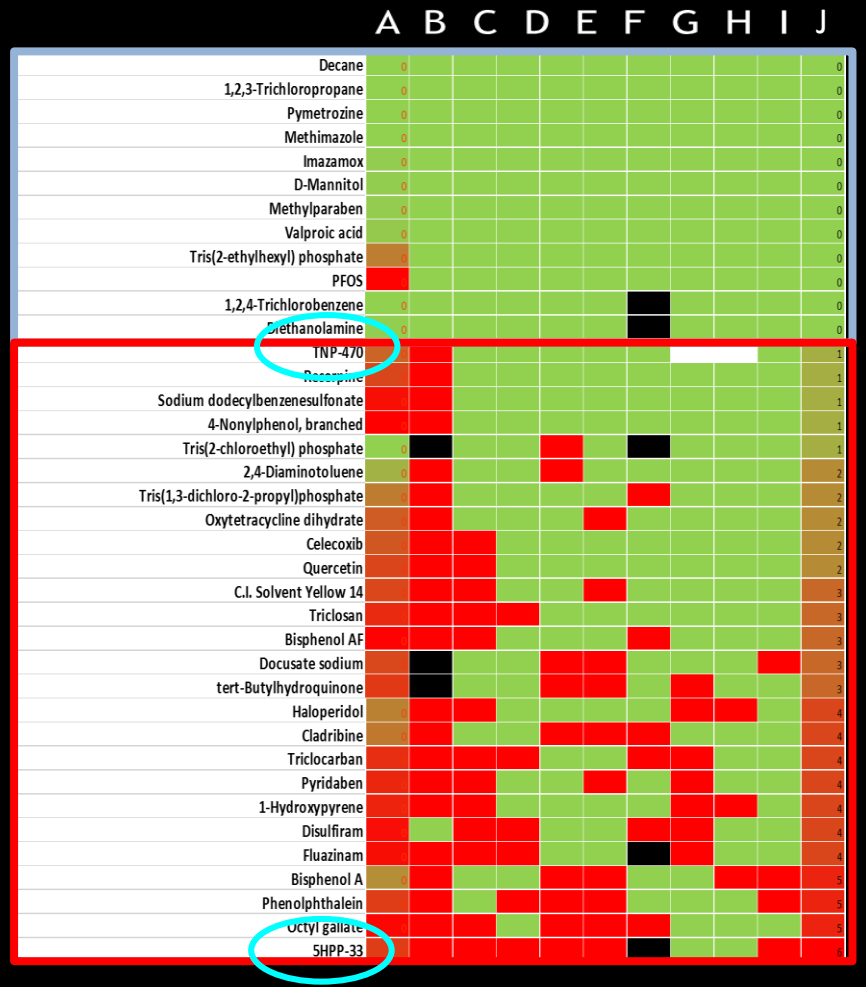








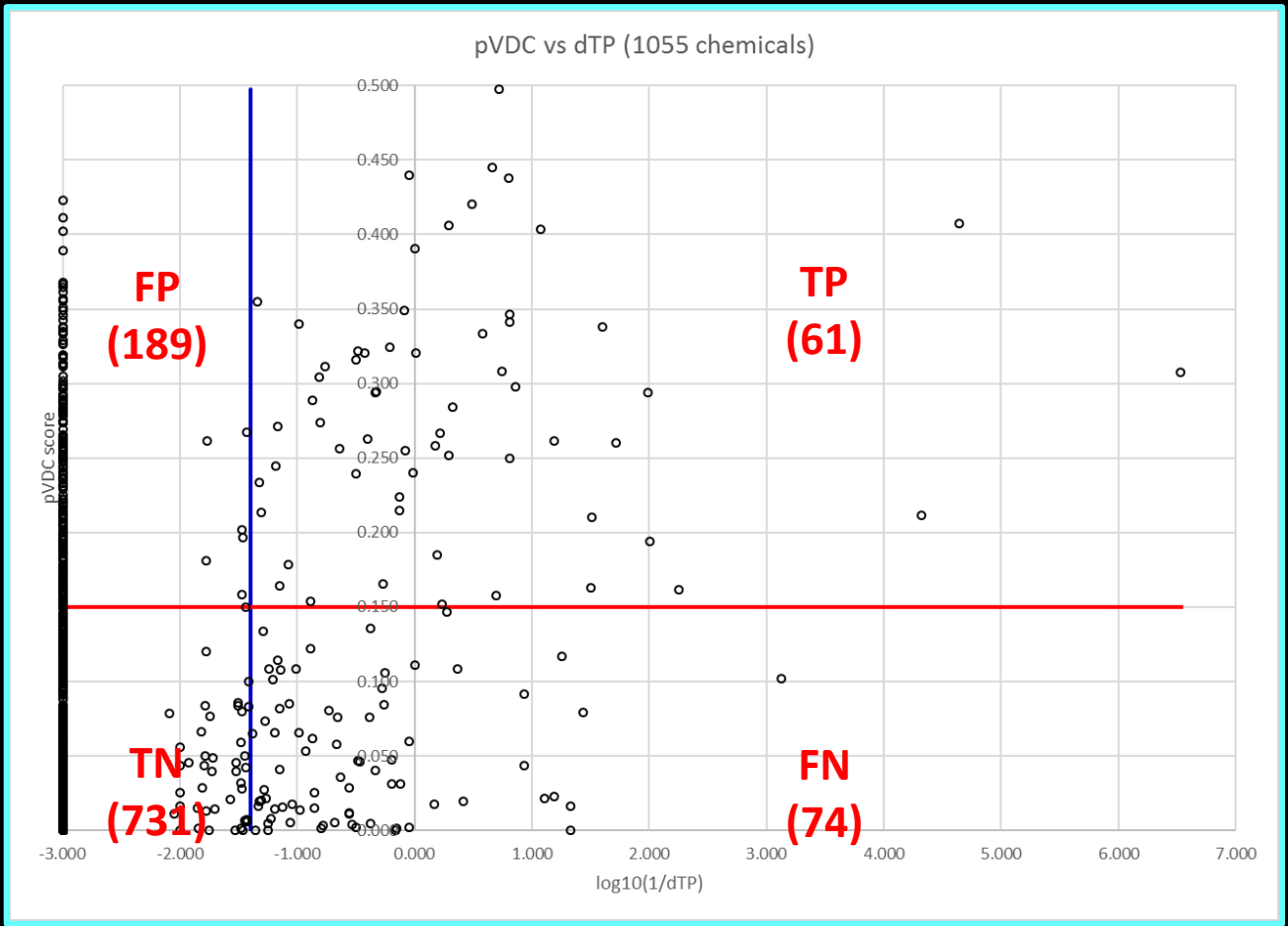




A ToxPi [1]
B FICAM tubulogenesis [2]
C Synthetic tubulogenesis [3]
D Matrigel tubulogenesis [3]
E nuCTNB [4]
F EC migration [4]
G angiogenic sprouting [5]
H TG-zebrafish [1]
I Vala tubulogenesis [2]
J aggregate (B to I)

[1] Tal et al. Reprod Toxicol (in press); [2] Knudsen et al., in prep; [3] Nguyen et al. Nature Bioengineering (in revision); [4] Belair et al. (2016) Acta Biomaterialia.

How well does pVDC score match ToxCast_Stemina predicted teratogenicity?



AOP-based **pVDC** score vs **DevTox** potential from the STM platform

(87-91% predictive of teratogenicity in a hES-based system)

Balanced Accuracy = 75.1%

(modeled on a 38-chem test set)

24.4% pVDC(+) also STM(+)

90.8% pVDC(-) also STM(-)

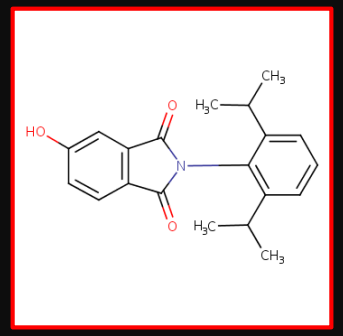
Computer Simulation of Developmental Processes and Toxicities (#3076):

SOT symposium 'Novel In Vitro and In Silico Platforms for Modeling Developmental and Reproductive Toxicity' [ITS]

Wednesday afternoon

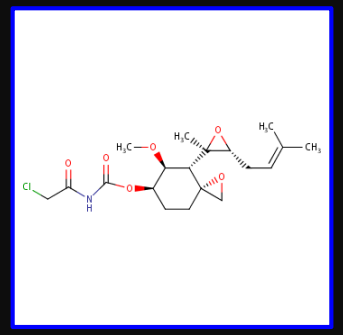
Reference Angiogenesis Inhibitors

5HPP-33

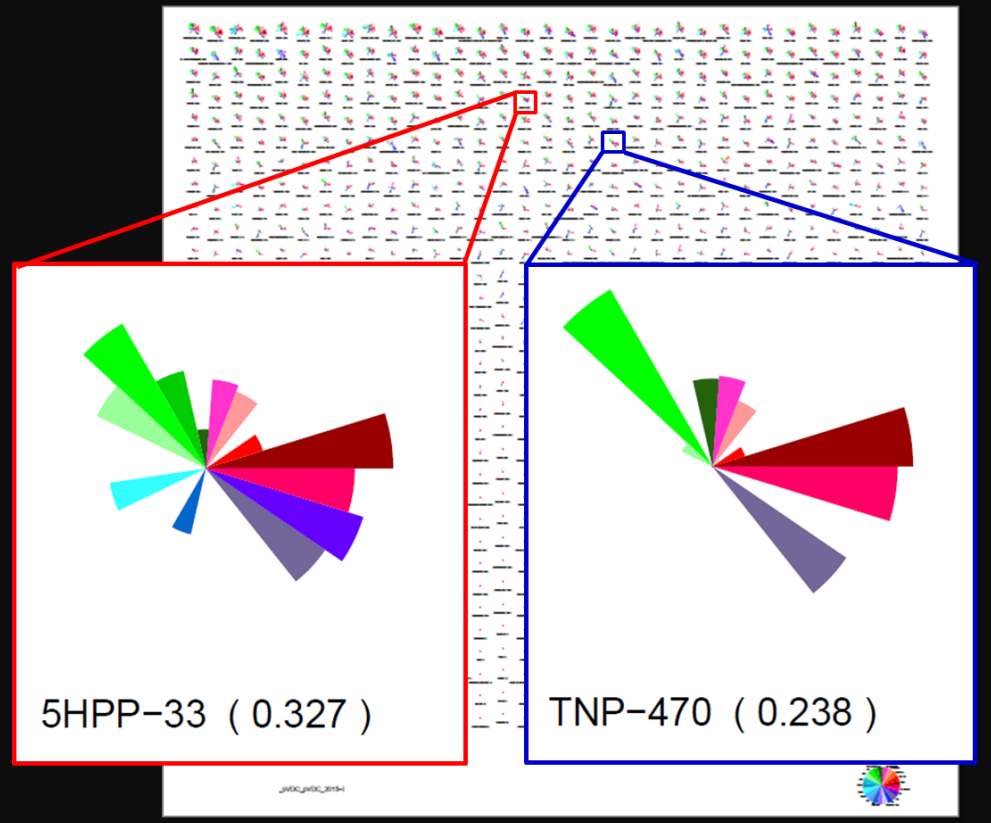


Synthetic thalidomide analog, destabilizes the tubulin network and disrupts endothelial tubulogenesis [Noguchi et al. 2005].

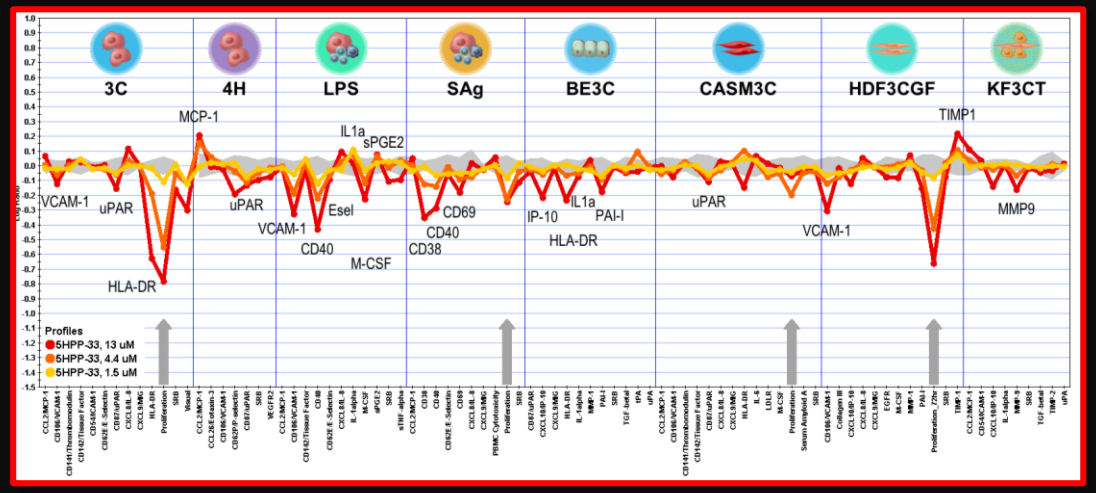
TNP-470

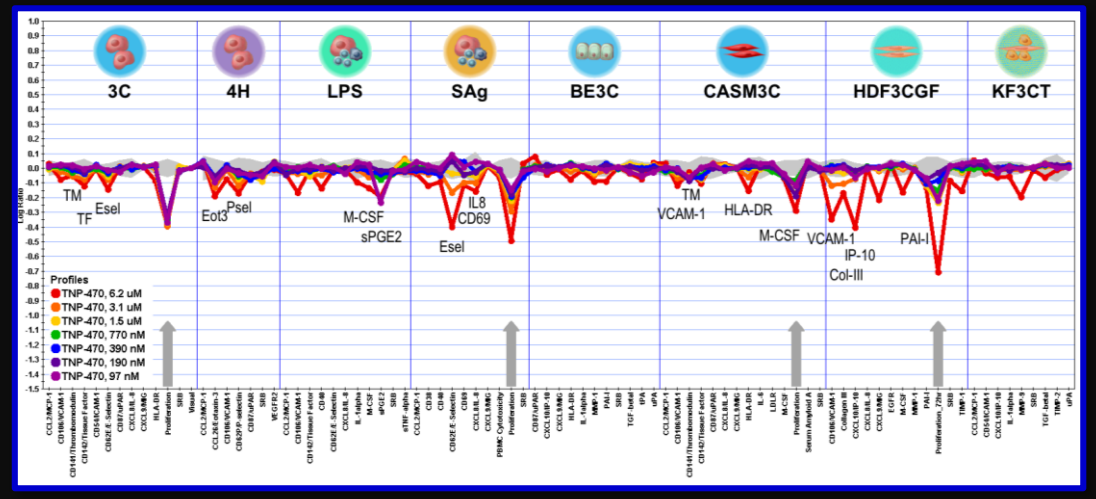


Synthetic fumagillin analog, inhibits MetAP2 and disrupts endothelial proliferation in response to Wnt signals [Griffith et al. 1998].



BioMAP database: Top BioSeek Reference Database Matchesfor human cell co-culture systems (inflammatory, vascular, …)





5HPP-33:

- profile match to Colchicine (microtubule disrupter R=0.844) and Docetaxel (microtubule stabilizer R=0.82)
- SVM match to mTOR inhibitor (4.4 uM), microtubule disrupter (13.0 uM), Mitochondrial inhibitor (40 uM).

TNP-470:

- profile match to GSK-461364A (PLK-1 inhibitor R=0.809) and BAY 11-7085 (IKβ alpha stabilizer R=0.797)
- SVM match to mTOR inhibitor (conc. In range 0.15 -1.5 uM).

Ellen Berg, DiscoverX-BioSeek

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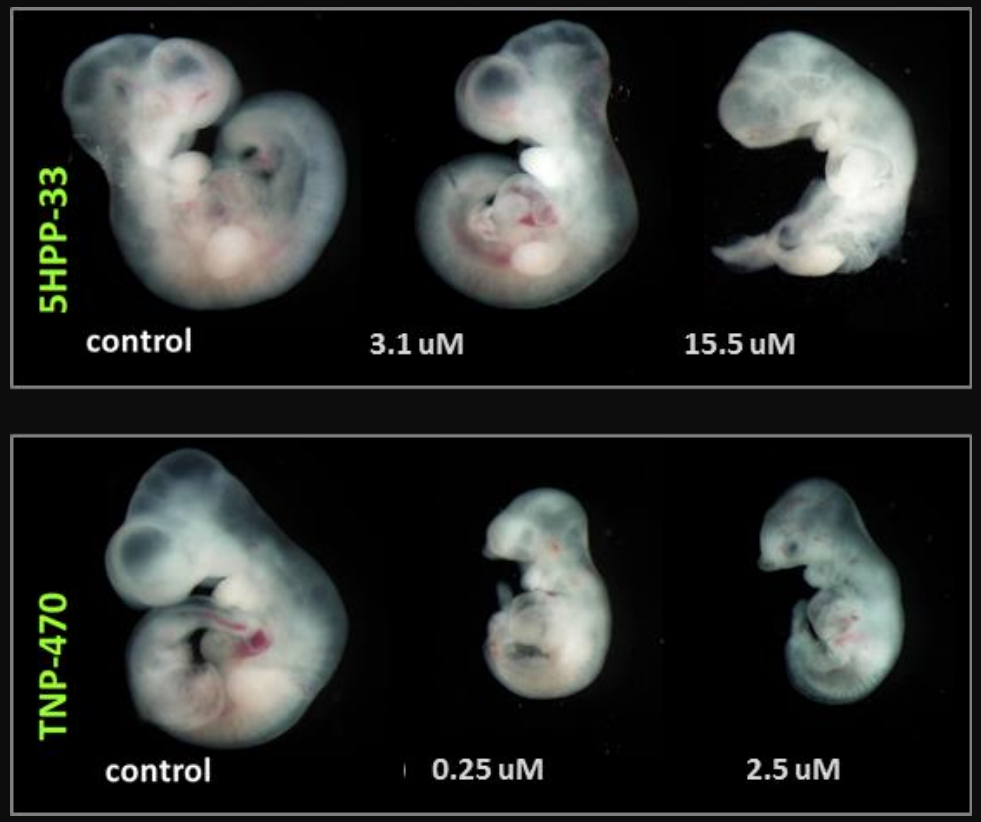
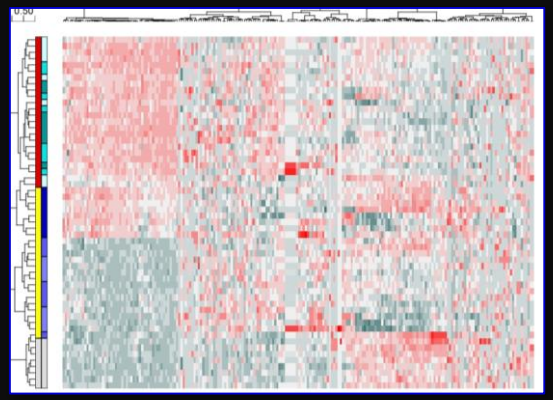
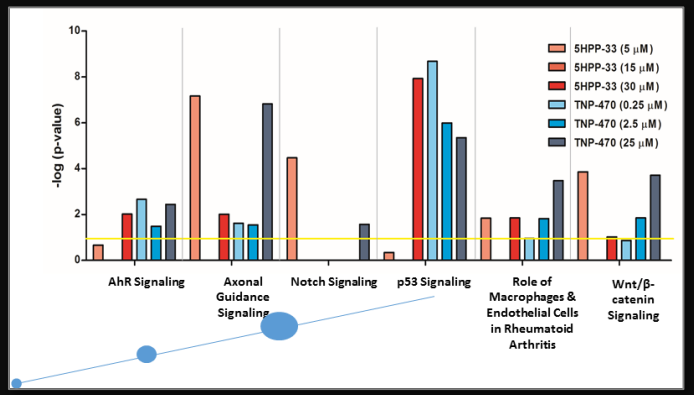
Rat WEC: GD10 embryos exposed for 48h



SOURCE: Ellis-Hutchings et al. (submitted)





RNA-seq analysis: p53 was most significantly altered pathway in both cases (5HPP-33, TNP-470); alterations in Notch and Wnt expression unique to 5HPP-33.

SOURCE: Franzosa et al. (in preparation)

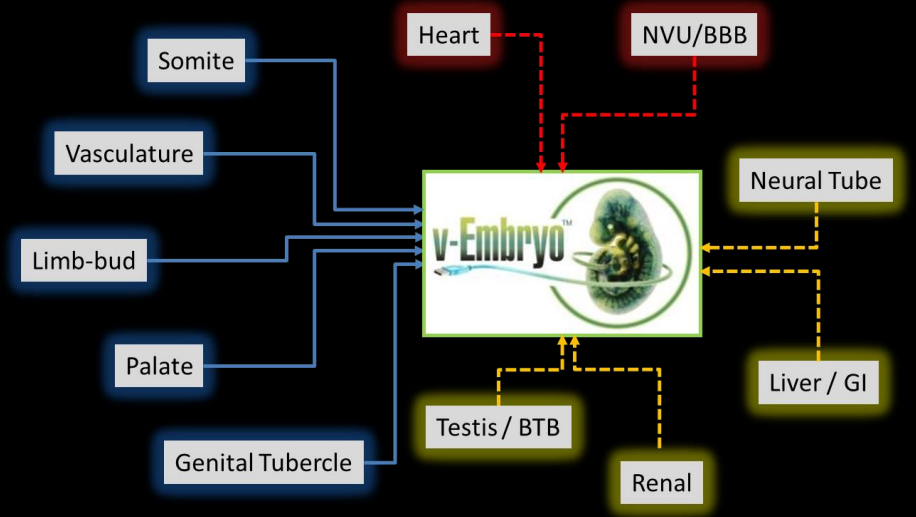
Tiered Testing Approach: to evaluate mechanistic drivers of vascular DevTox

ASSAY	READOUT (uM)	HPP-33	TNP-470
FICAM tubulogenesis	AC50	0.67	2.2
Rat AEA	AC50	1.3	0.018
ArunA hNP migration/prolif	AC20	1.7	---
Tox21 p53 induction	AC50	2.6	>17.4
ZFISH embryotox (DOW)	AC50	3.4	0.032
BSK BioMAP	mTOR inhibition	4.4	0.15
STM viable cells	50% loss	7.1	5.2
STM targeted biomarker	<0.88 ORN/CYSS	9.5	0.01
VALA endothelial migration	nuCTNB	10.0	---
VALA tubulogenesis	inhibition	16.7	---
ToxCast TCB	median AC50	16.7	2.4
Rat WEC quality	AC50	21.2	0.038

Breathing life into a ‘Virtual Embryo’

- **Hypothesis:** computer models that recapitulate a morphogenetic series of events can be used analytically (to understand) and theoretically (to predict) developmental toxicity.

- **Agent-Based Modeling and Simulation (ABMS):** a heuristic approach to reconstruct tissue dynamics from the bottom-up, cell-by-cell and interaction-by-interaction.

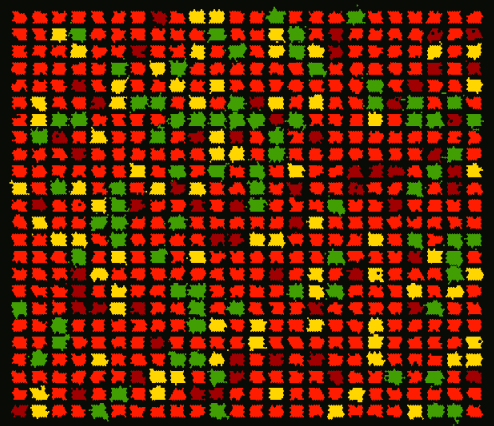


In Silico Dynamics: Computer Simulation in a Virtual Embryo (#3117):
SOT symposium ‘Quantitative Systems Toxicology for Chemical Safety Assessment’ [ITS]
Thursday morning

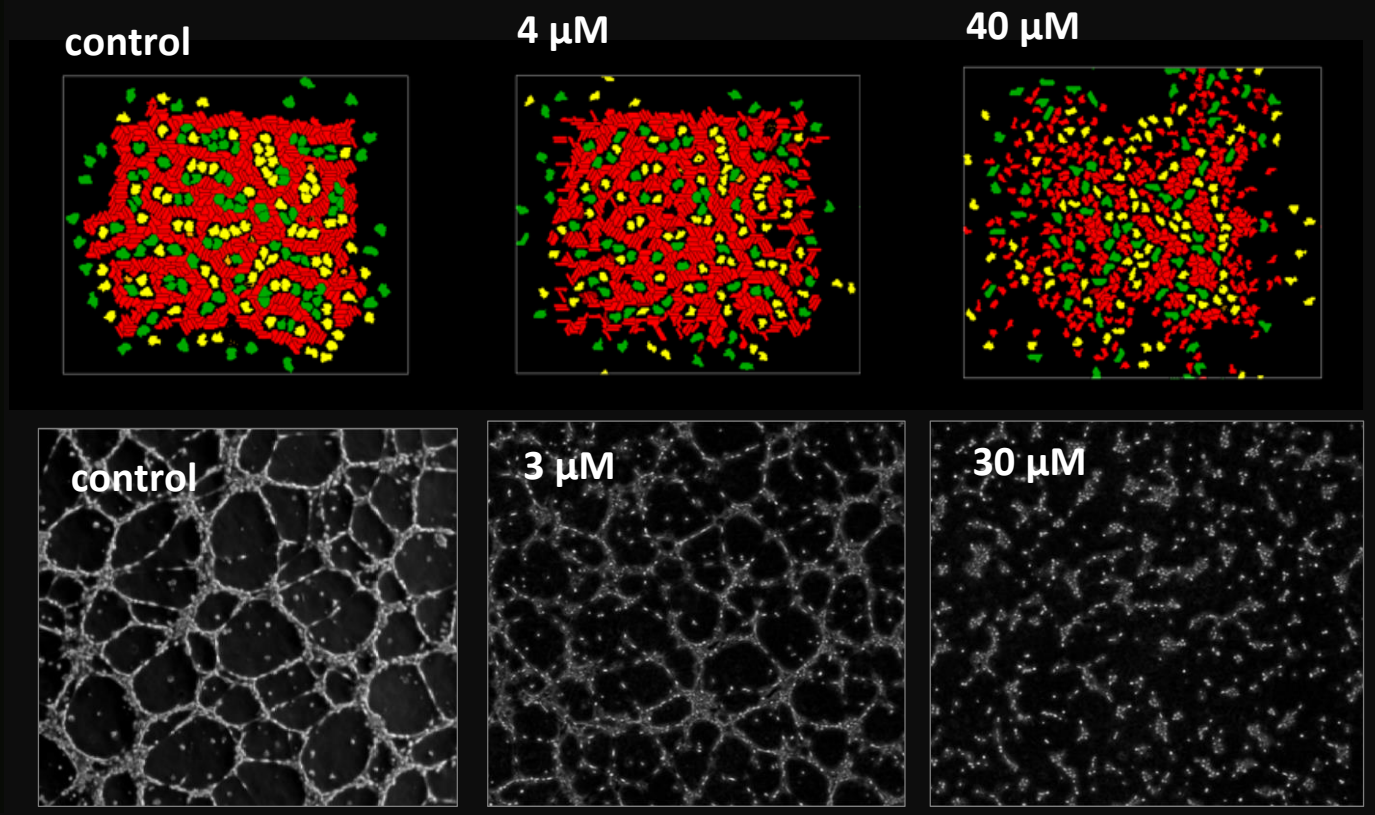
Simulated Angiogenesis: cell agent-based model in compucell3d

VEGF165
MMPs
VEGF121
sFlit1
TIE2
CXCL10
CCL2

ABMS



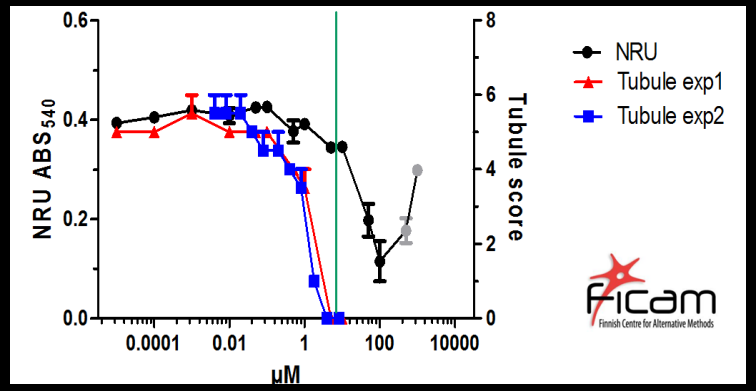
Endothelial Stalk
Endothelial Tip
Mural Cell
Inflammatory Cell

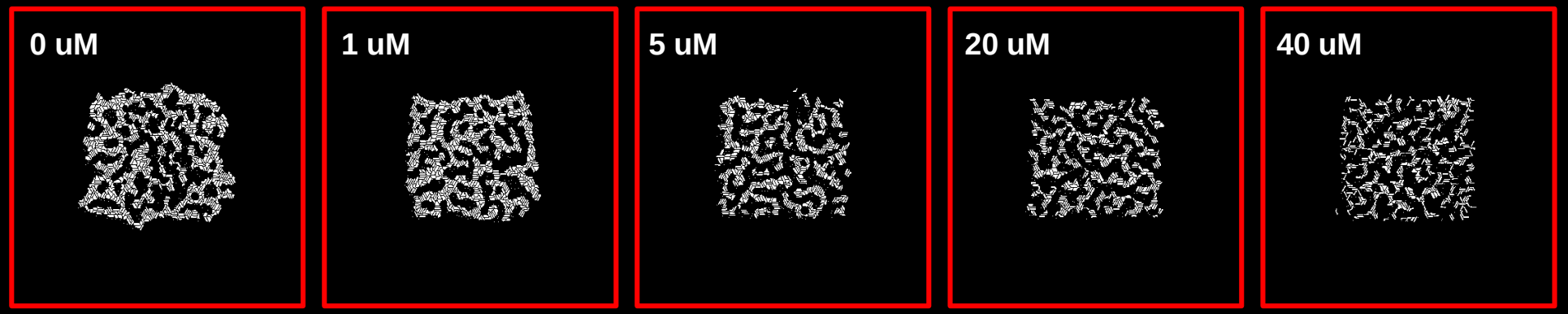


Exposure to 5HPP-33

SOURCE: Kleinstreuer et al. 2013, PLoS Comp Biol

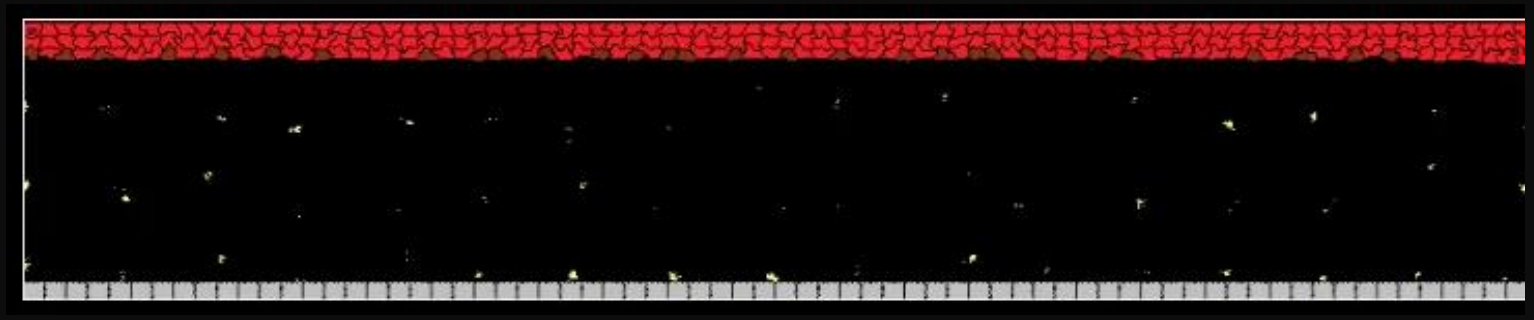
Octyl Gallate

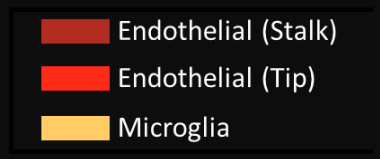


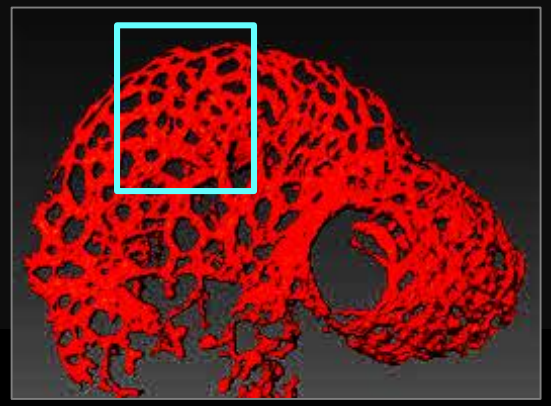


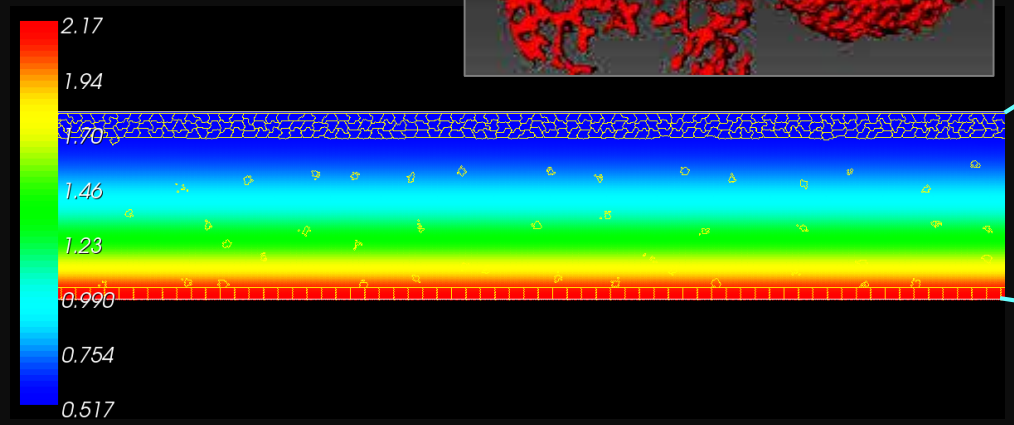
vascular disruption *in silico*

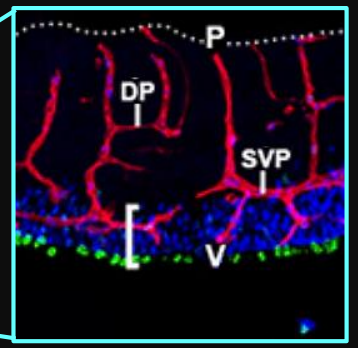
Brain angiogenesis





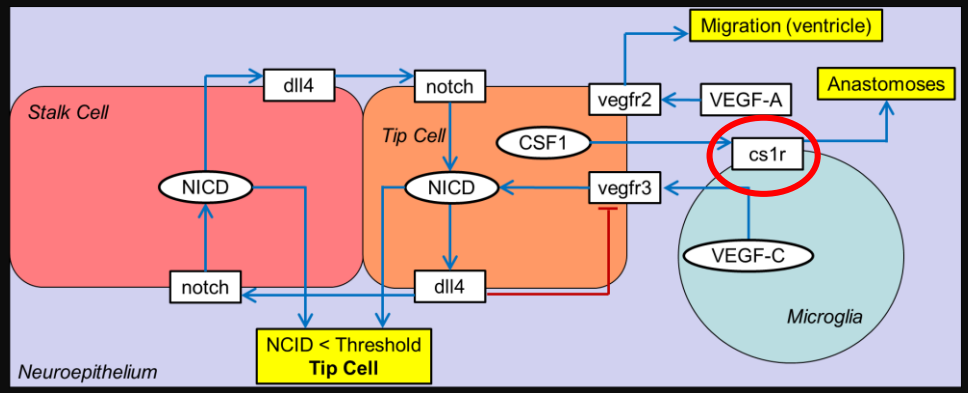






Tata et al., *2016,* PNAS

- PNVP sprouting angiogenesis vascularizes the neuroepithelium.
- Vascularity is increased by microglia, the resident macrophages of the CNS.
- Microglial cell growth and survival is directly tied to CSF1R signaling.



T Zurlinden, K Saili – NCCT (2017)

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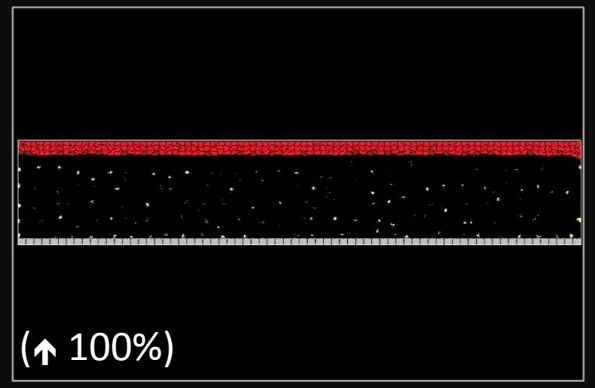
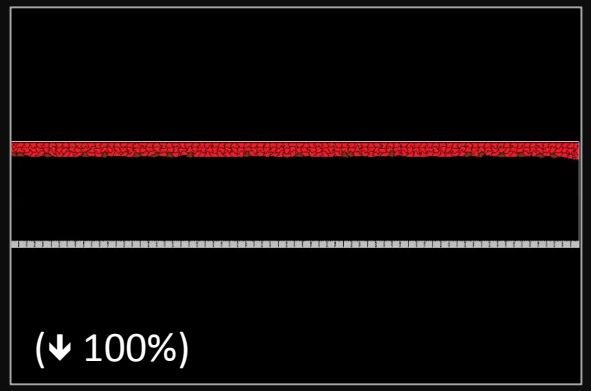
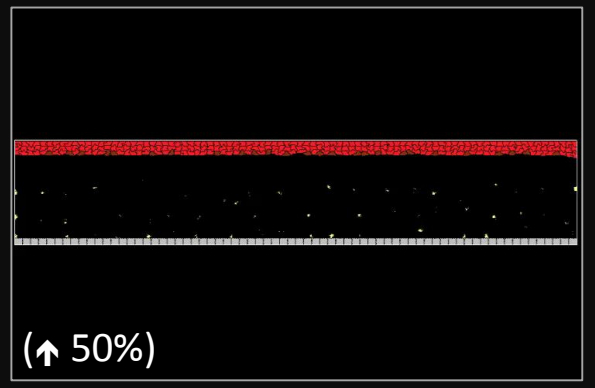
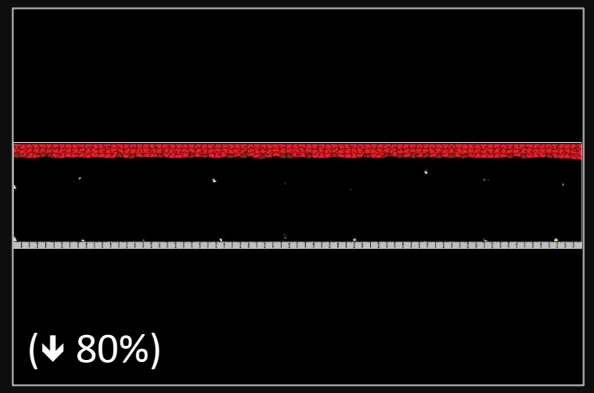
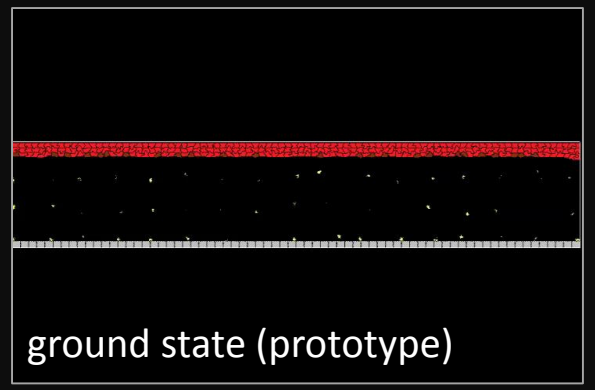
Quantitative Response: vascular complexity *vs* microglia abundance

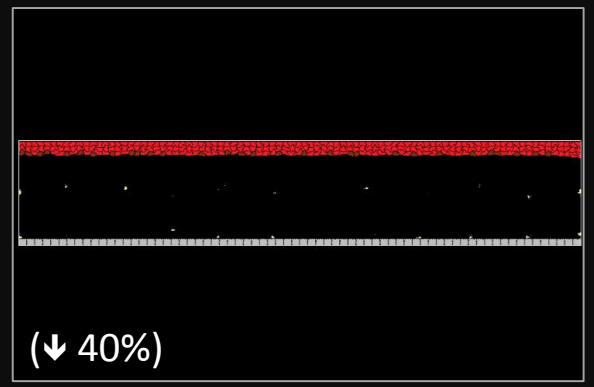




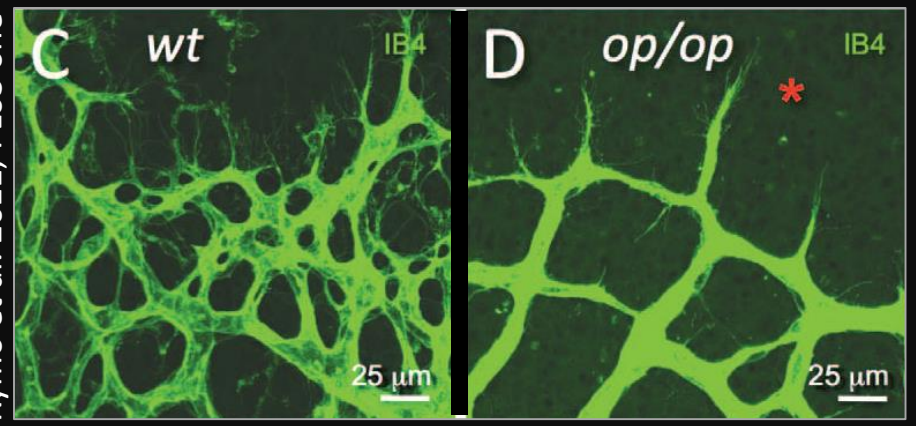


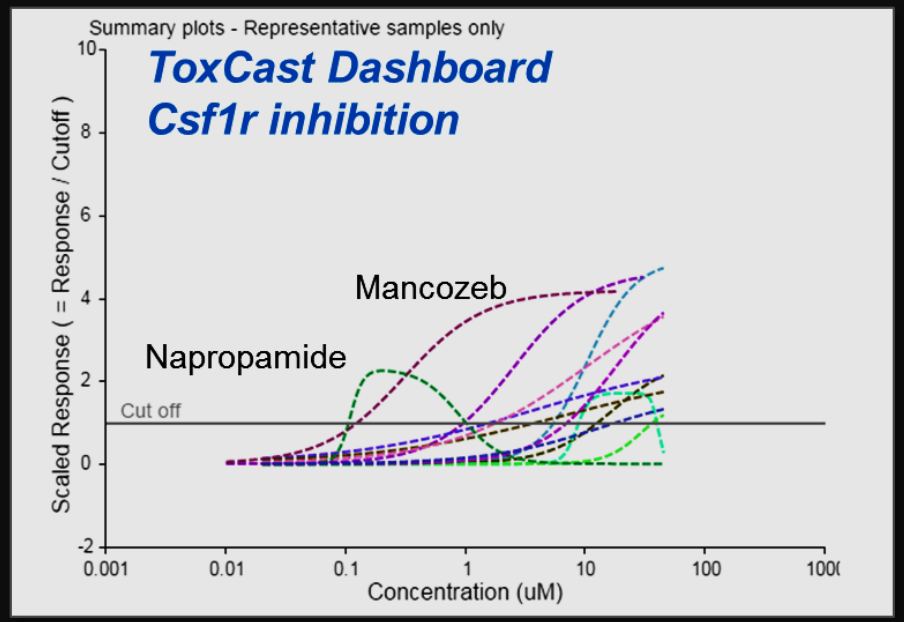






Rymo et al. 2011, PLoS one





RELEVANCE and APPLICATION

- Intended use of this AOP in a regulatory context is predictive toxicology of developmental hazards, especially for integrating data from HTS assays into cell agent-based models for predicting dysmorphogenesis.

- As part of an integrated assessment of toxicity, AOP can identify useful information for assessing AOs relevant to risk assessment and efficient use of resources for validation through predictive models linking developmental toxicity to vascular disruption.

- AOP-based computer models that simulate vascular development can usher-in new virtual screening techniques to predict what might happen to a developing embryo when exposed to chemicals across different dose-time-stage scenarios, including the range of effects and how cellular injury propagates across development.

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http://www2.epa.gov/sites/production/files/2015-08/documents/virtual_tissue_models_fact_sheet_final.pdf