NTP
National Toxicology Program

Embryonic Vascular Disruption and Adverse Prenatal Outcomes

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ILS/NICEATM

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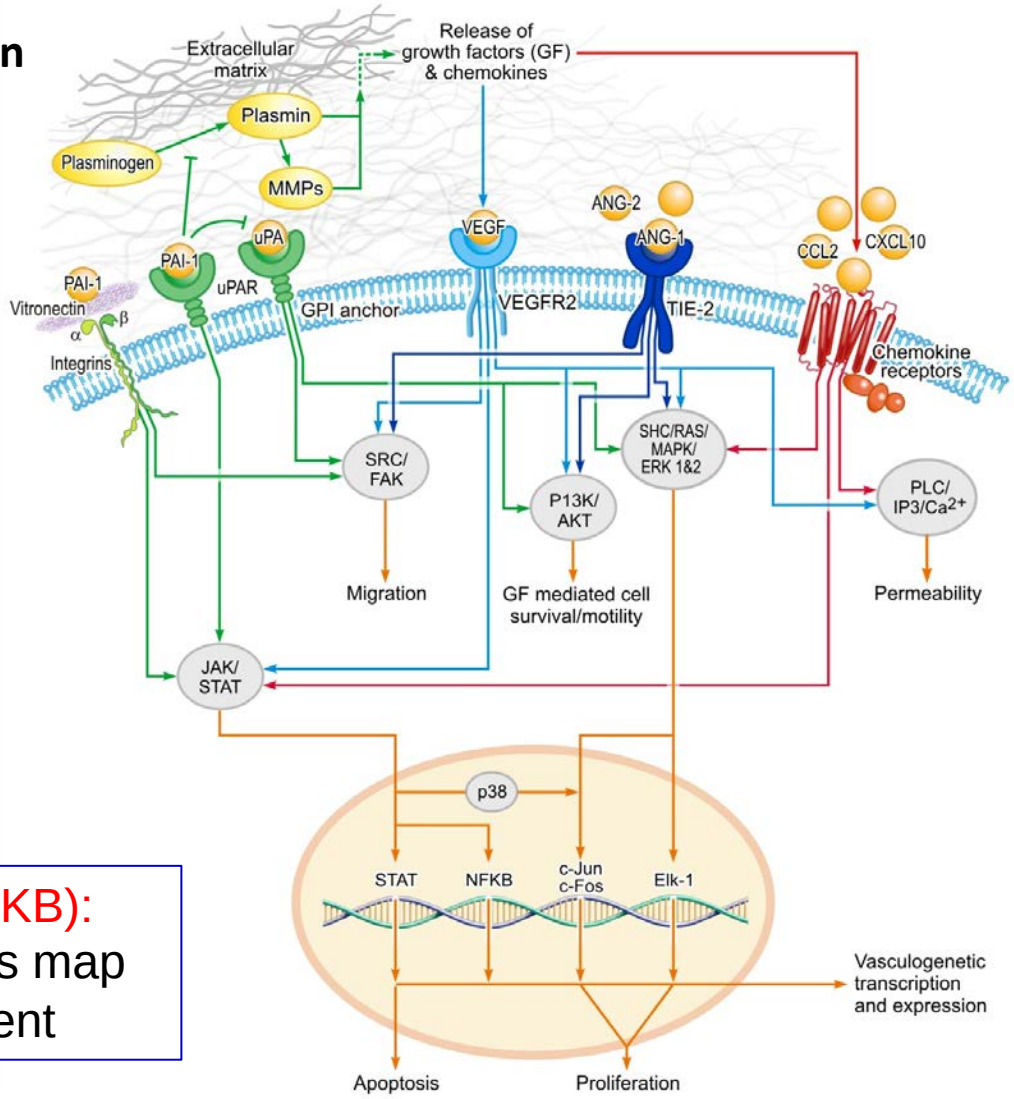
3 September 2014

Tox21

DEPARTMENT OF HEALTH & HUMAN SERVICES • USA

Vascular Developmental Processes

- **endothelial proliferation & cell migration**
  - growth factors
  - chemokine signaling
- **extracellular matrix degradation**
  - plasminogen activating system
  - matrix metalloproteinases
- **neovascular stabilization**
  - Ang/Tie2 signaling
  - vascular remodeling



Virtual Tissues-Knowledge Base (VT-KB):
~100 distinct ToxCastDB assay targets map to key systems in vascular development

SOURCE: Kleinstreuer et al. 2011, Env Hlth Persp 119: 1596-1603

ToxCastDB: 700+ HTS Assays

Assay Provider
- ACEA
- Apredica
- Attagene
- BioSeek
- CellzDirect
- NCGC/Tox21
- NHEERL MESC
- NHEERL NeuroTox
- NHEERL Zebrafish
- NovaScreen
- Odyssey Thera

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

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-living embryos

Species
- Human
- Rat
- Mouse
- Zebrafish
- Sheep
- Boar
- Rabbit
- Cattle
- Guinea pig

Tissue Source

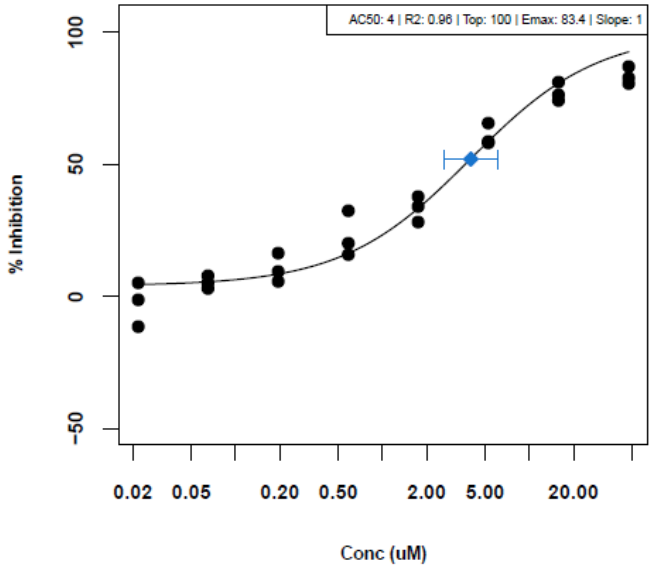
Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

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

(http://actor.epa.gov/actor/faces/ToxCastDB)

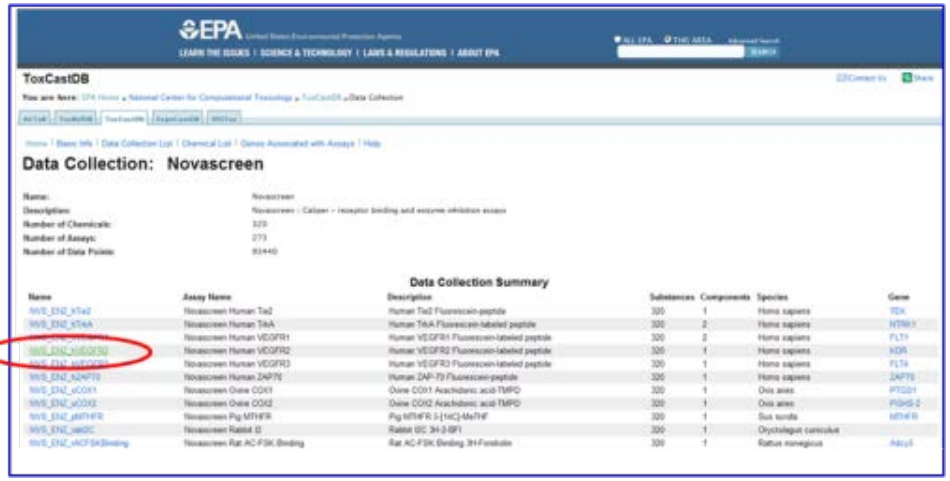
ToxCastDB

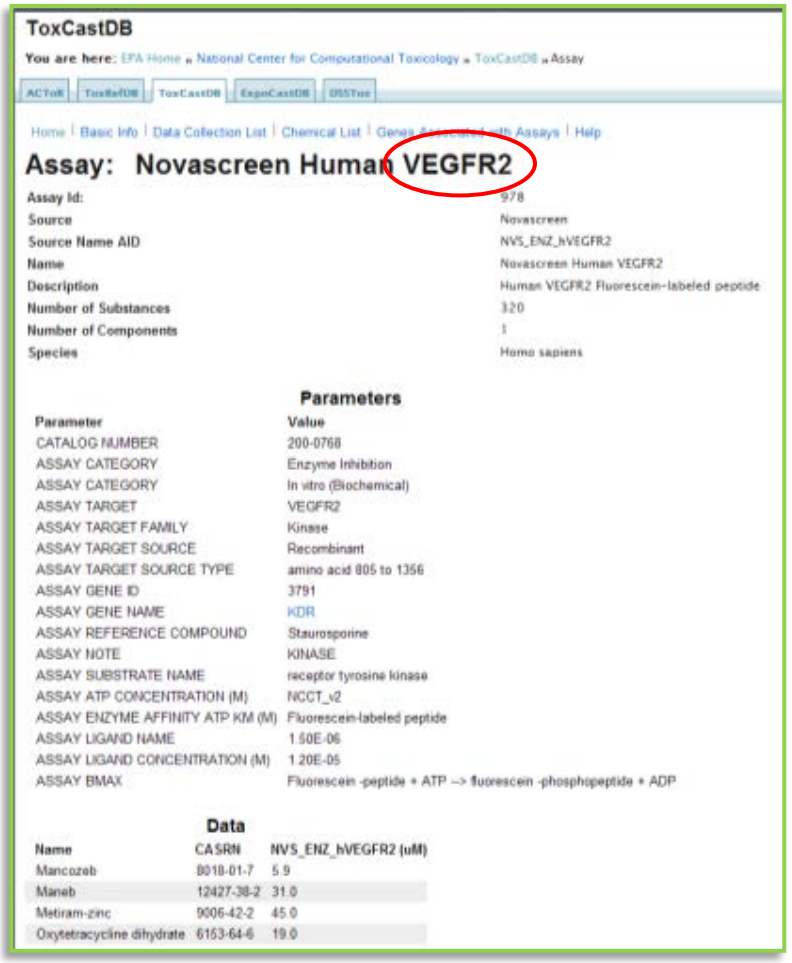
http://actor.epa.gov/actor/faces/ToxCastDB/DataCollection.jsp



AC50 concentration producing a 50% change

LEC lowest effect concentration





- 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

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Environ Health Perspect. 2011 Nov;119(11):1596-603. doi: 10.1289/ehp.1103412. Epub 2011 Jul 25.

Environmental impact on vascular development predicted by high-throughput screening.

Kleinstreuer NC, Judson RS, Reif DM, Sipes NS, Singh AV, Chandler KJ, Dewoskin R, Dix DJ, Kavlock RJ, Knudsen TB

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Author information

Abstract

BACKGROUND: Understanding he
diverse chemical landscape and pa
Agency (EPA) ToxCast™ project pr
in vitro assays in phase I (complete
diverse biological targets and build
developmental health and disease

Birth Defects Research, Part C

Embryo Today: Reviews

Review

Disruption of embryonic vascular development in predictive toxicology†

Thomas B. Knudsen*, Nicole C. Kleinstreuer

Issue

Article first publis
DOI: 10.1002/bd
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A Computational Model Predicting Disruption of Blood Vessel Development

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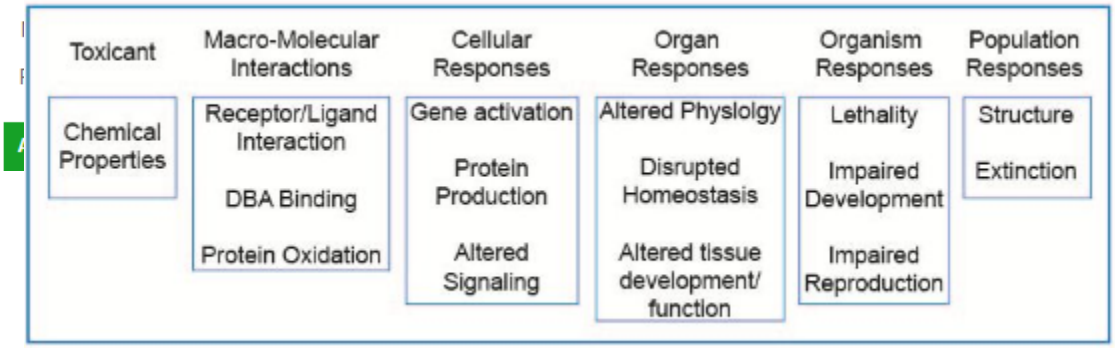
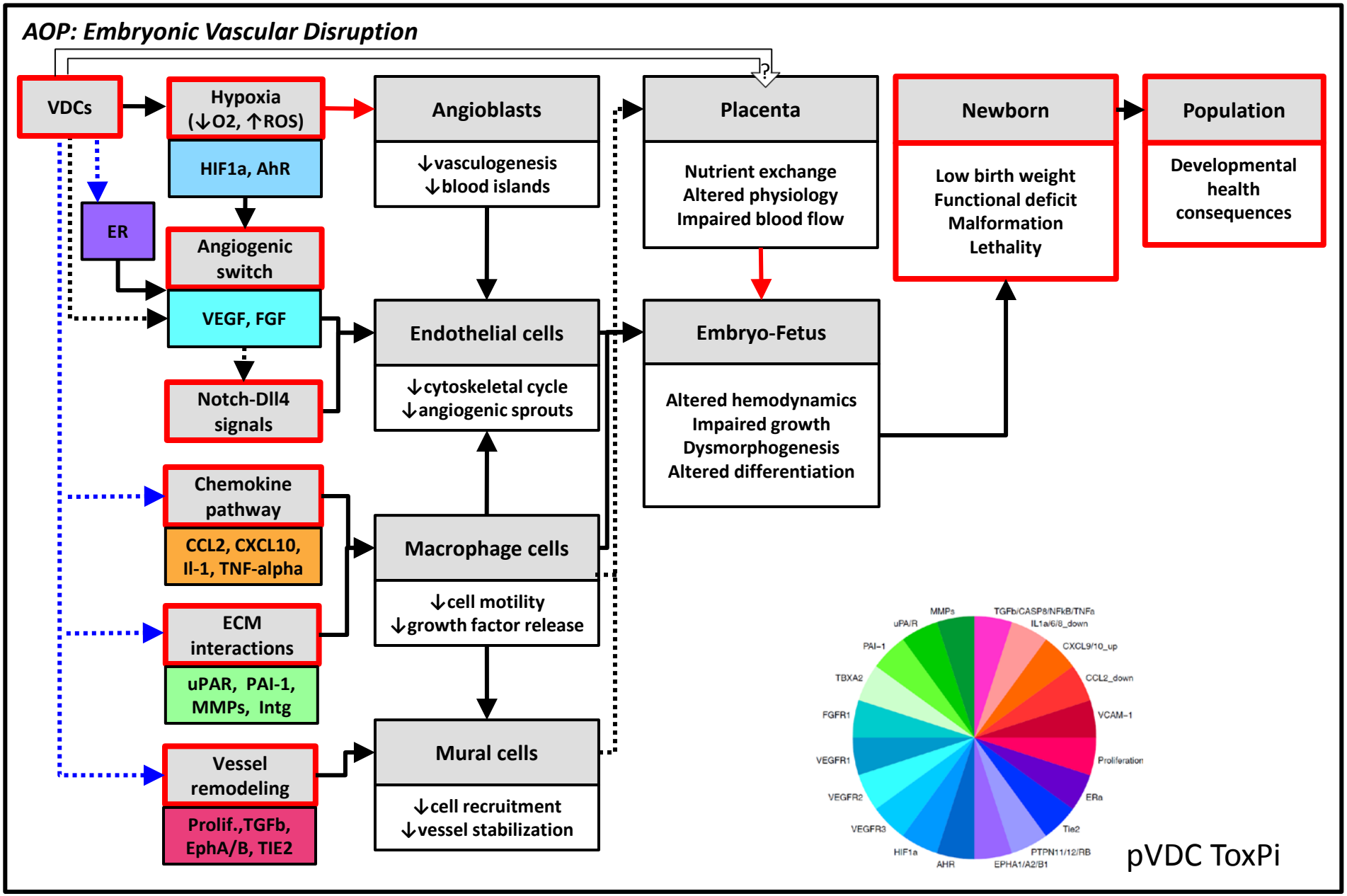


Adverse Outcome Pathway Framework



Established mechanistic linkage with quantitative or semi-quantitative data

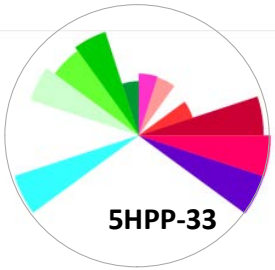
Predictive model linkages based on quantitative concentration-response data

Test the pVDC signature

Developmental vascular toxicity predictive signature

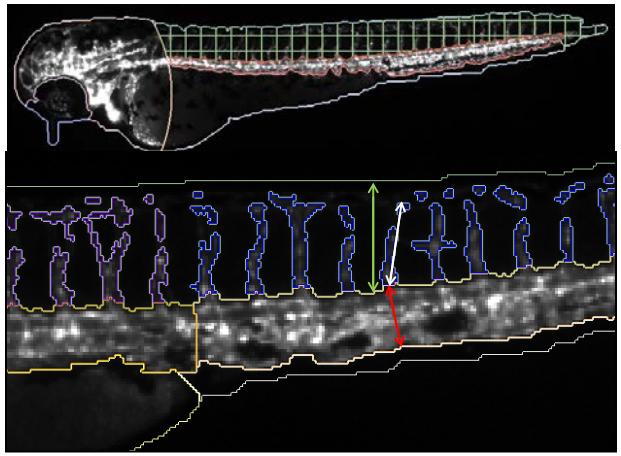
Rank order 1060 ToxCast compounds based on putative ability to disrupt developmental angiogenesis

ToxPi

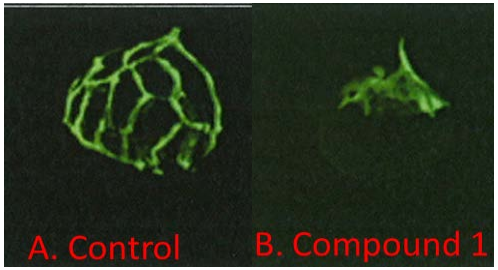


Thalidomide analog

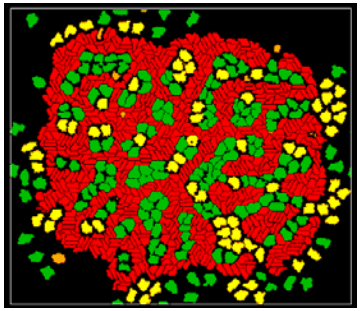
Zebrafish trunk vessel assay



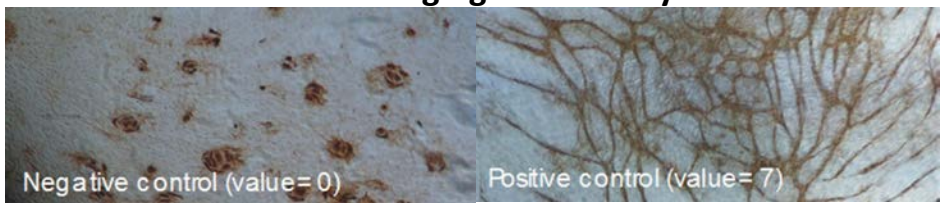
Zebrafish hyaloid vessel assay



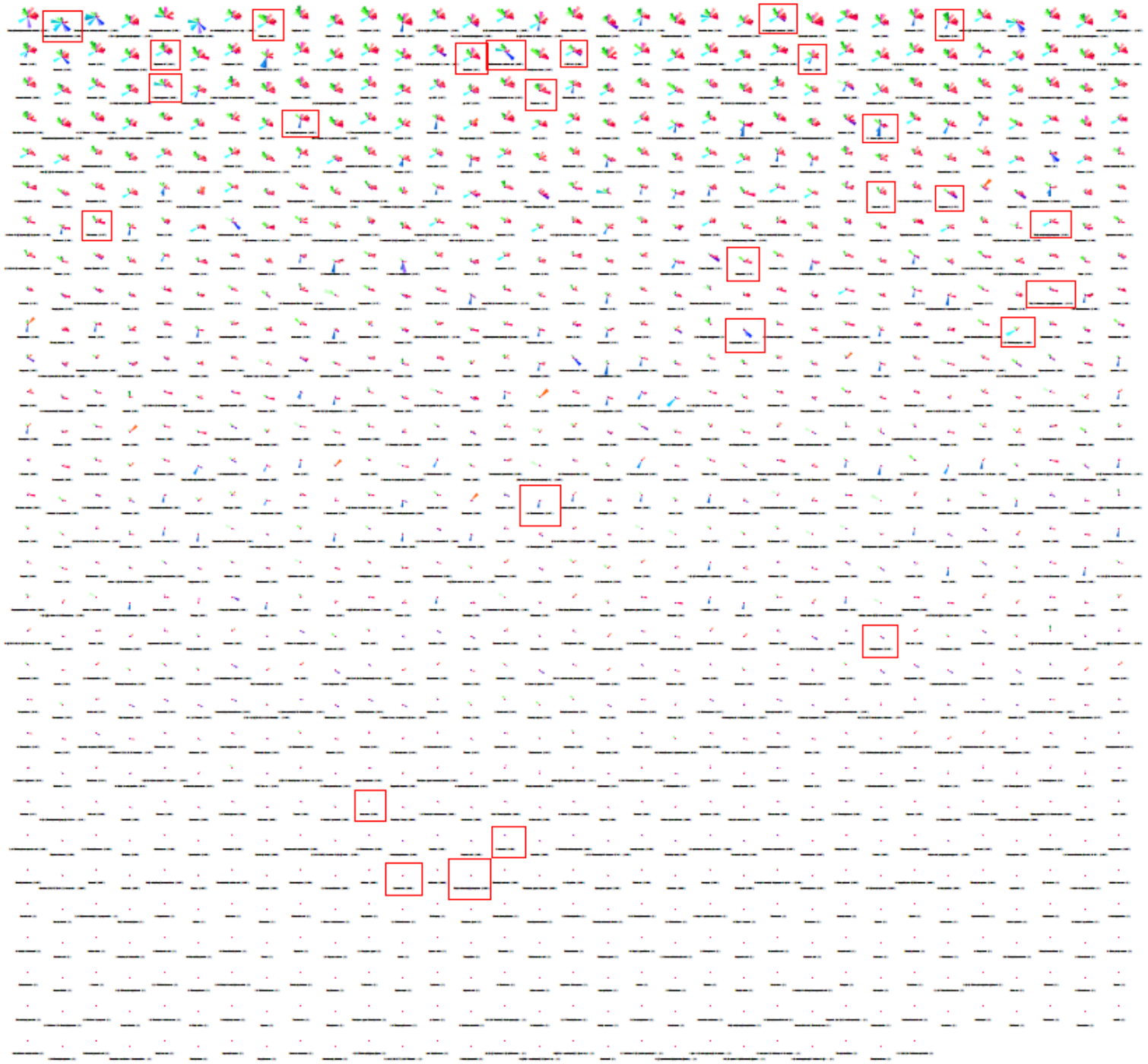
***in silico* model of vascular plexus formation**



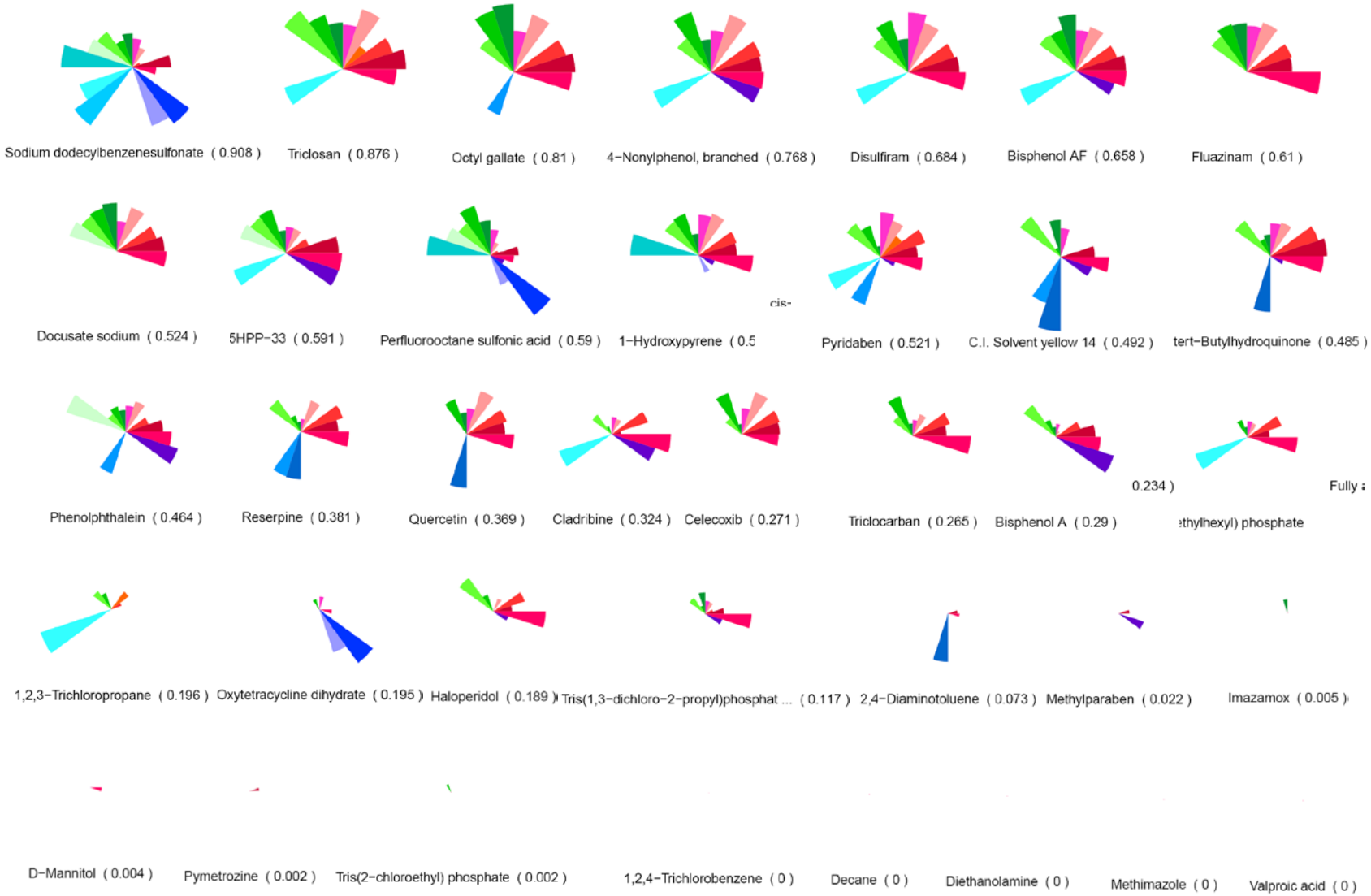
Human angiogenesis assay



Poster I-4-550:
Human cell-based functional 3D-angiogenesis test for identification of inhibitors of angiogenesis
T. Toimela, O. Huttala, J-R. Sarkanen, T. Heinonen



Chemical selection: Test the pVDC signature

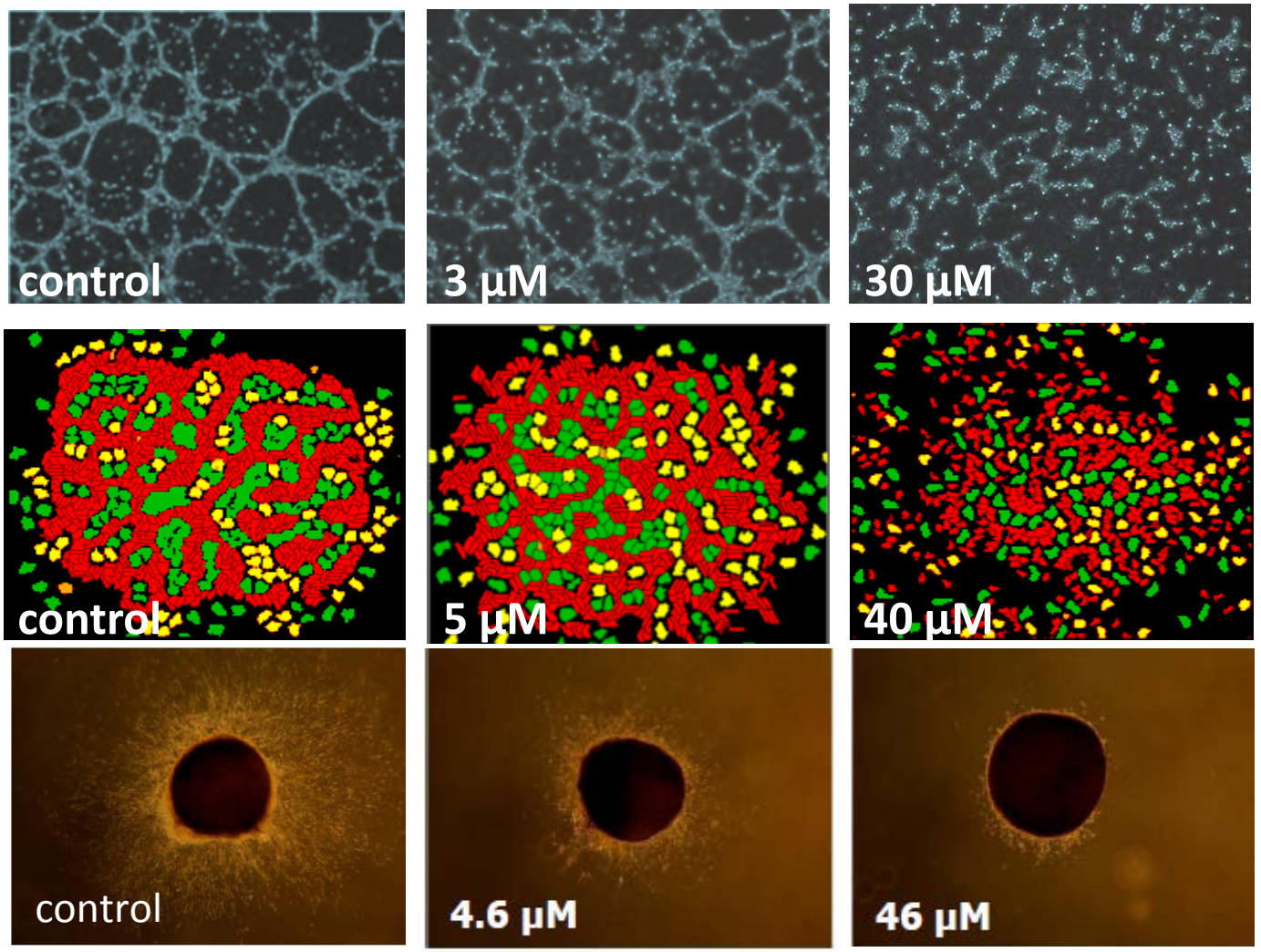


Validation of vascular disruption AOP by orthogonal assays:
in vitro, *in silico*, and *in situ*

In vitro
(HUVEC)
Noguchi et al. 2005, Bioorg Med Chem Lett.

In silico
(virtual tissue)
Kleinstreuer et al (2013) PLoS Comp Biol 9(4): e1002996

In situ
(Aortic explant)
Carney & Ellis-Hutchings, Dow Chemical Co. (manuscript in prep)



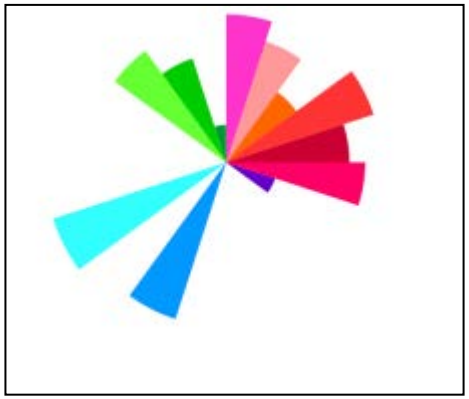
5HPP-33 exposure disrupts angiogenesis *in vitro*, *in silico*, and *in situ*

Virtual Tissues & Human Cell Based Tubulogenesis

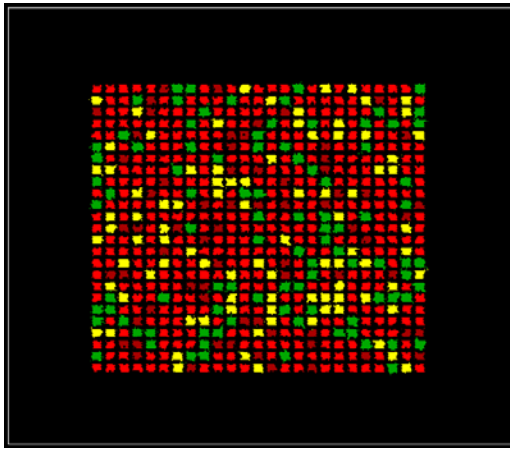
ToxCast prediction

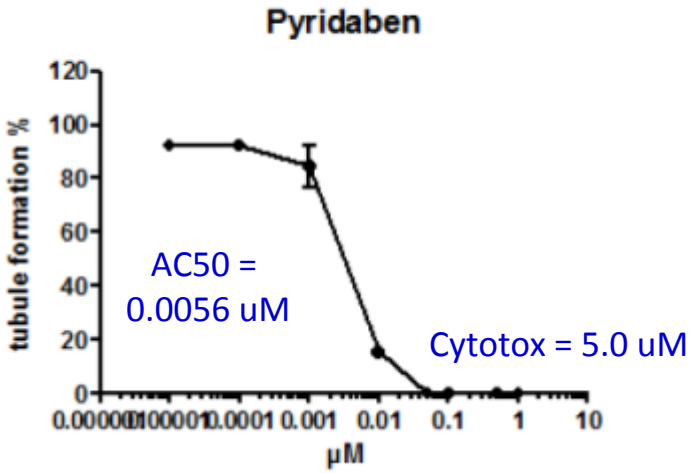
Virtual Tissue model

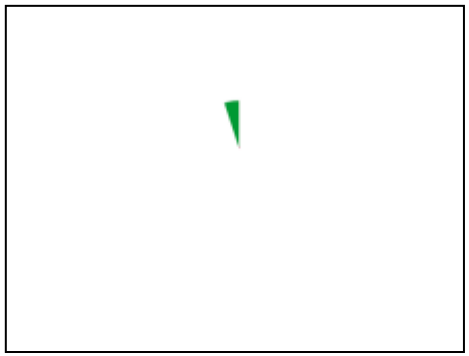
In vitro qualification



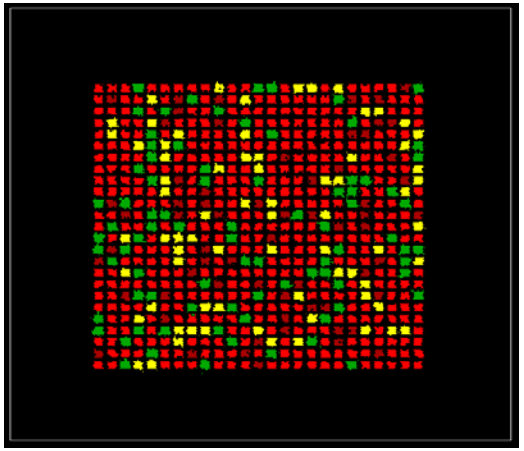
Pyridaben

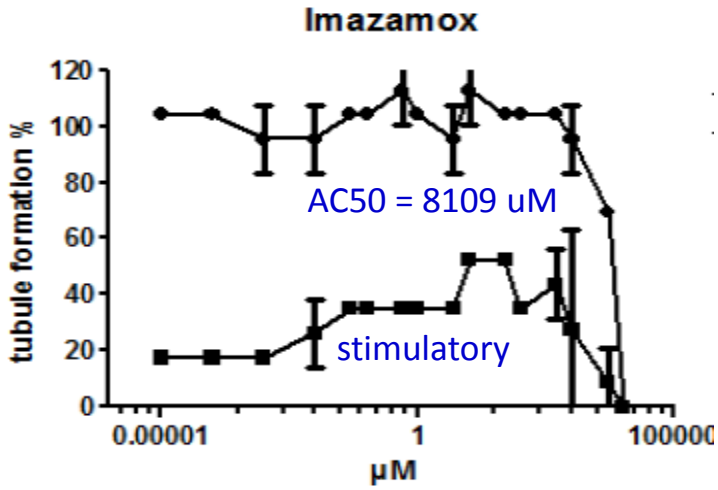






Imazamox

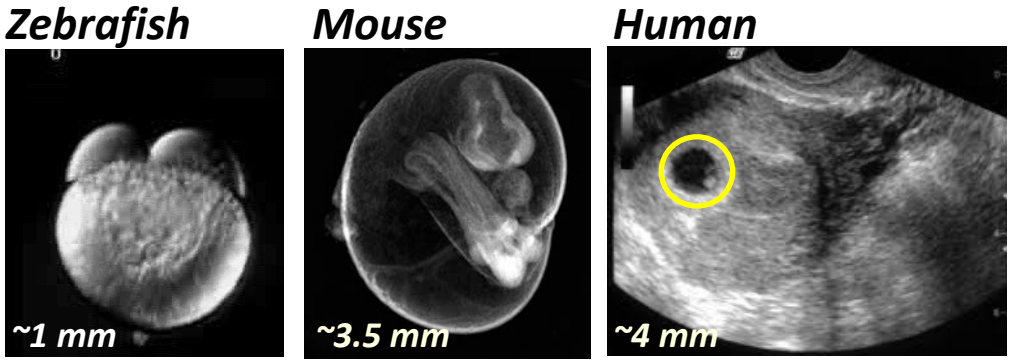


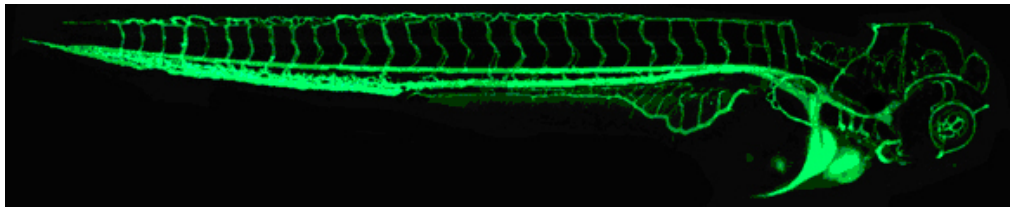


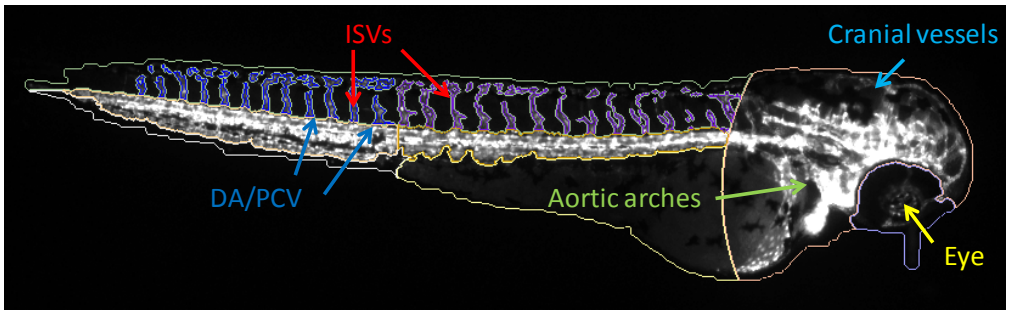
SOURCE: FICAM (T Heinonen and R Sarkanen)

Zebrafish embryogenesis: A Quantitative AOP Model?

- a biologically complex system
  - - vascular developmental toxicity
- conserved pathways
  - - 75% of genes have human homologs
- embryo is transparent
  - - amenable to quantitative imaging
- transgenic reporter lines
  - - map vasculature across space-time
- rapid and scalable platform
  - - amenable to automation and HTS

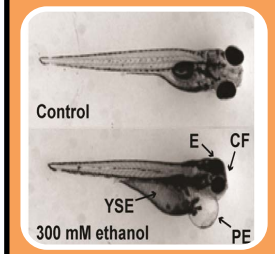






SOURCE: Tamara Tal, EPA/NHEERL-ISTD

Zebrafish trunk vessel assay
Screening strategy

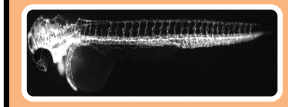


Define overt toxicity

- Start at 80 µM
- Semi-log spacing
- 8 concentrations
- n=1, 2 embryos/rep
- Chlopyrifos positive control (8 + 80 µM)

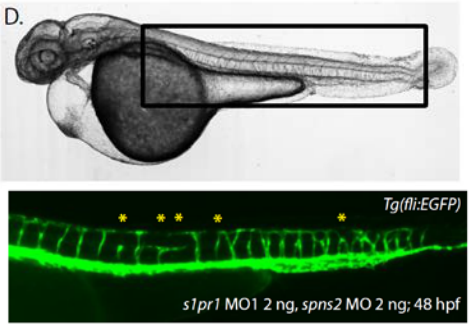
Criteria for inclusion

- <15% controls abnormal
- Positive controls on each plate

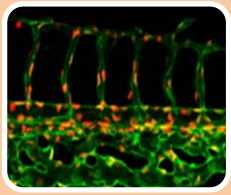


Screen for developmental vascular toxicity

- Start at LOEL
- Quarter-log spacing
- 4 concentrations
- n=2, 2 embryos/rep
- PTK787 positive control (4 µM)



Source: Mendelson et al. 2012.

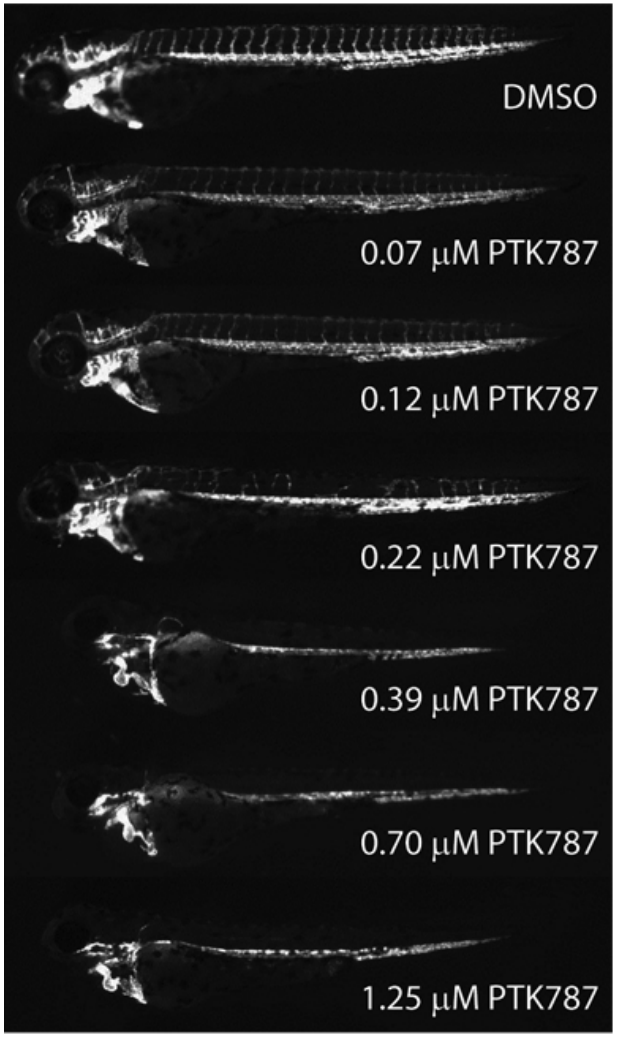


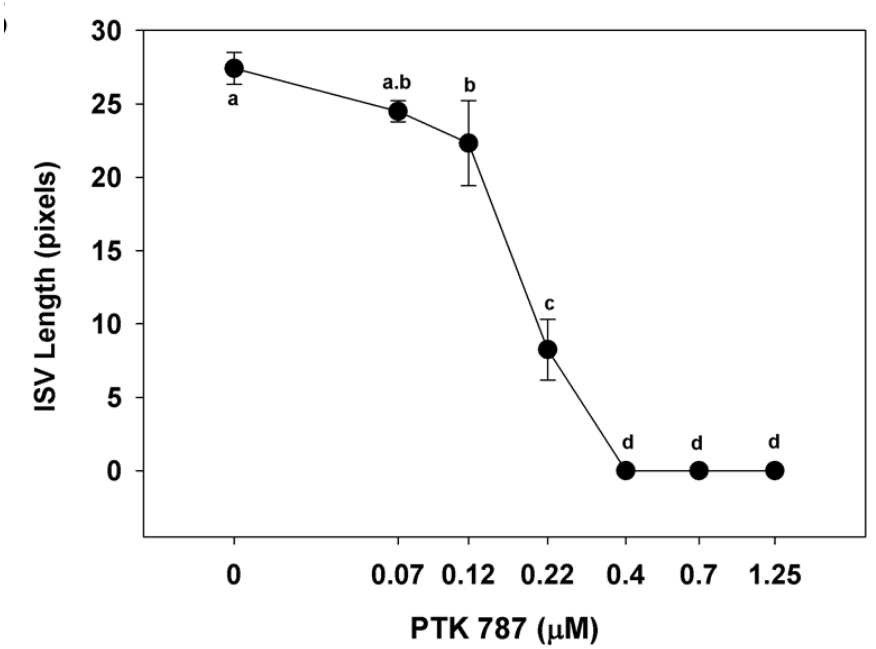
Quantify vascular toxicity

- For hits and subset of
- negative compounds
- Start at LOEL
- Quarter-log spacing
- 8 concentrations
- n=3, 2 embryos/rep
- PTK787 positive control (4 µM)

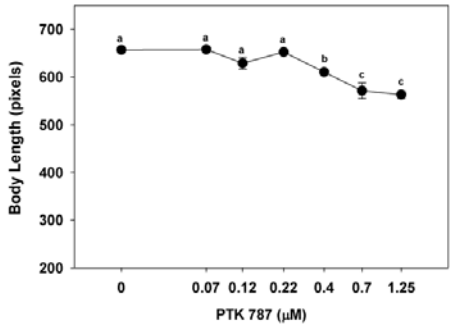
SOURCE: T. Tal, EPA/NHEERL-ISTD

Impaired angiogenesis in larvae exposed to PTK787 (VEGFR2 inhibitor)



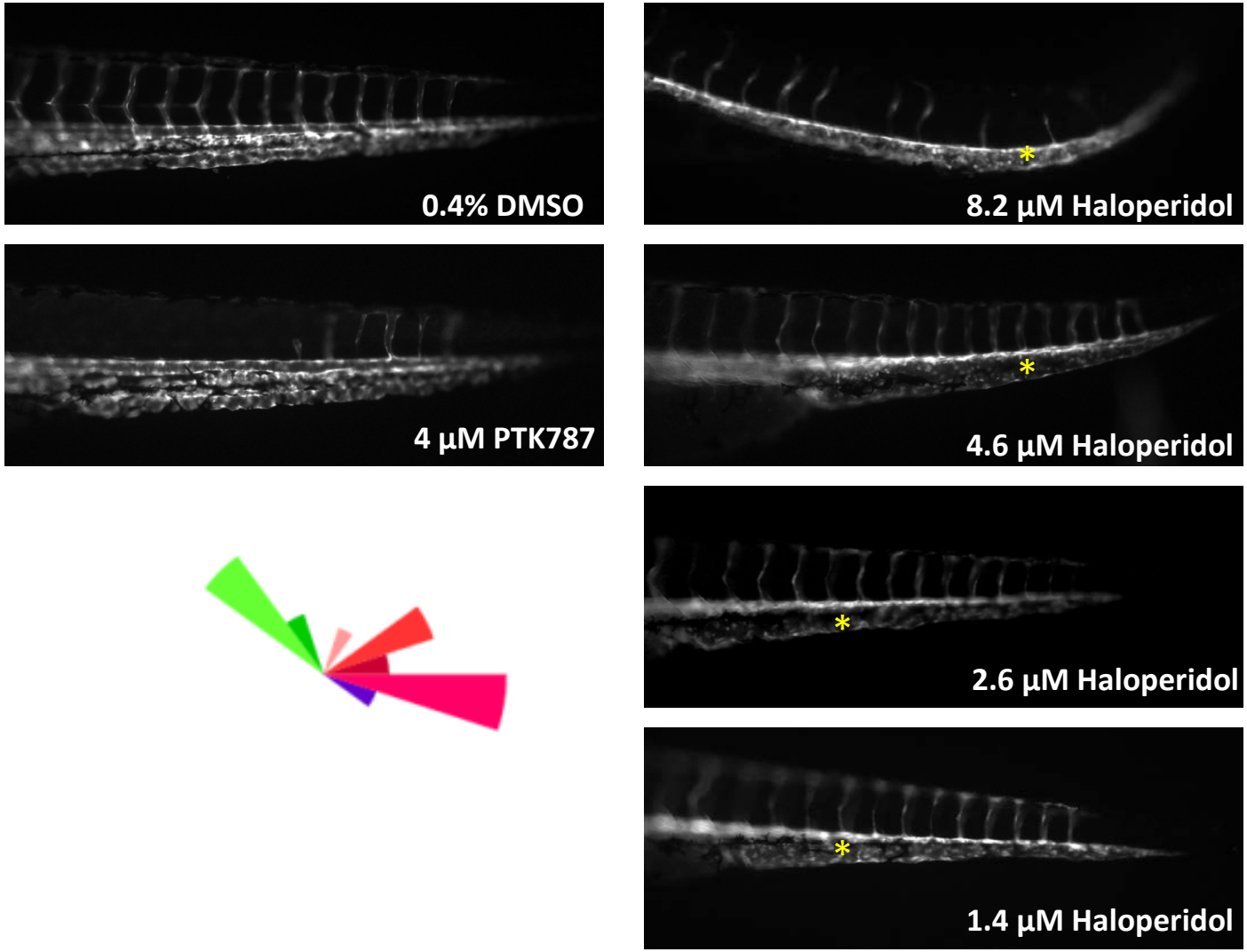


Overt Toxicity: (Body Length)



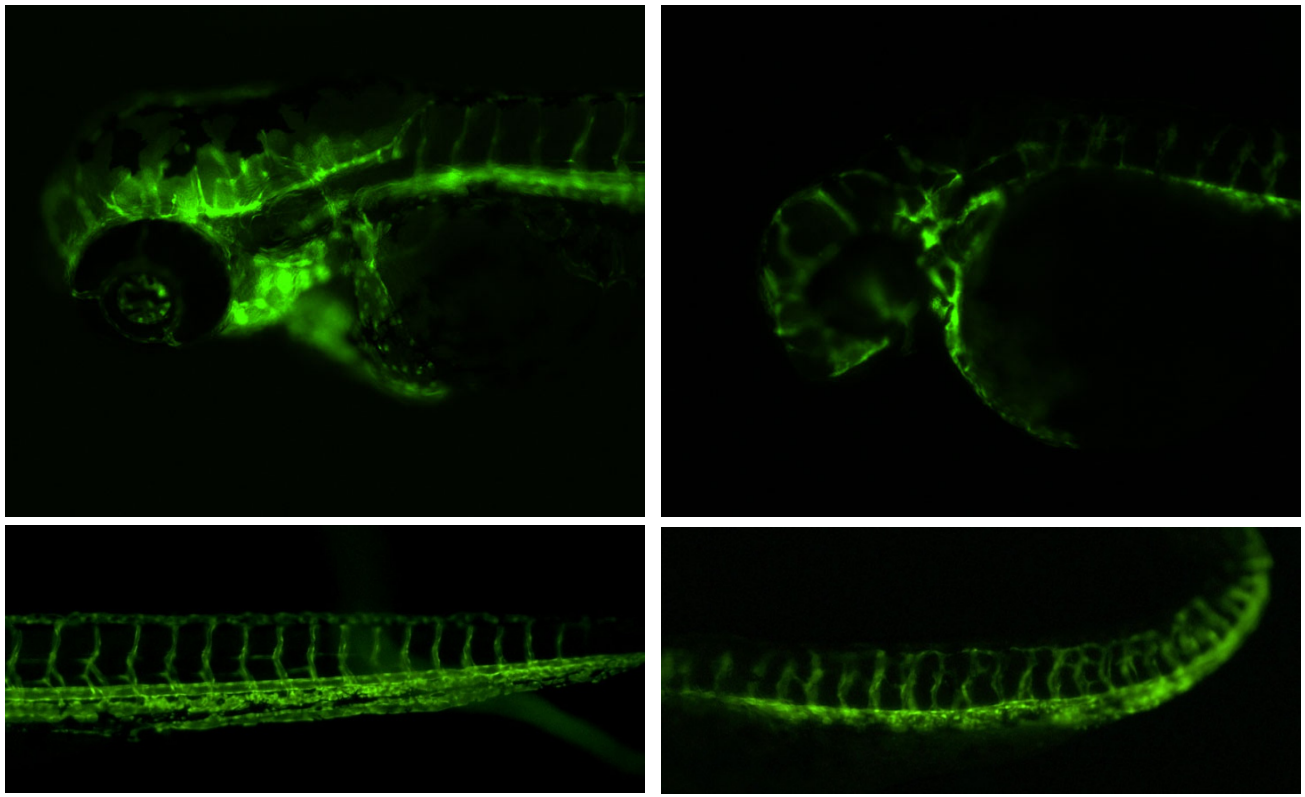
Source: Tal et al. 2014

Predominant CVP phenotype: Haloperidol



SOURCE: T. Tal, EPA/NHEERL-ISTD

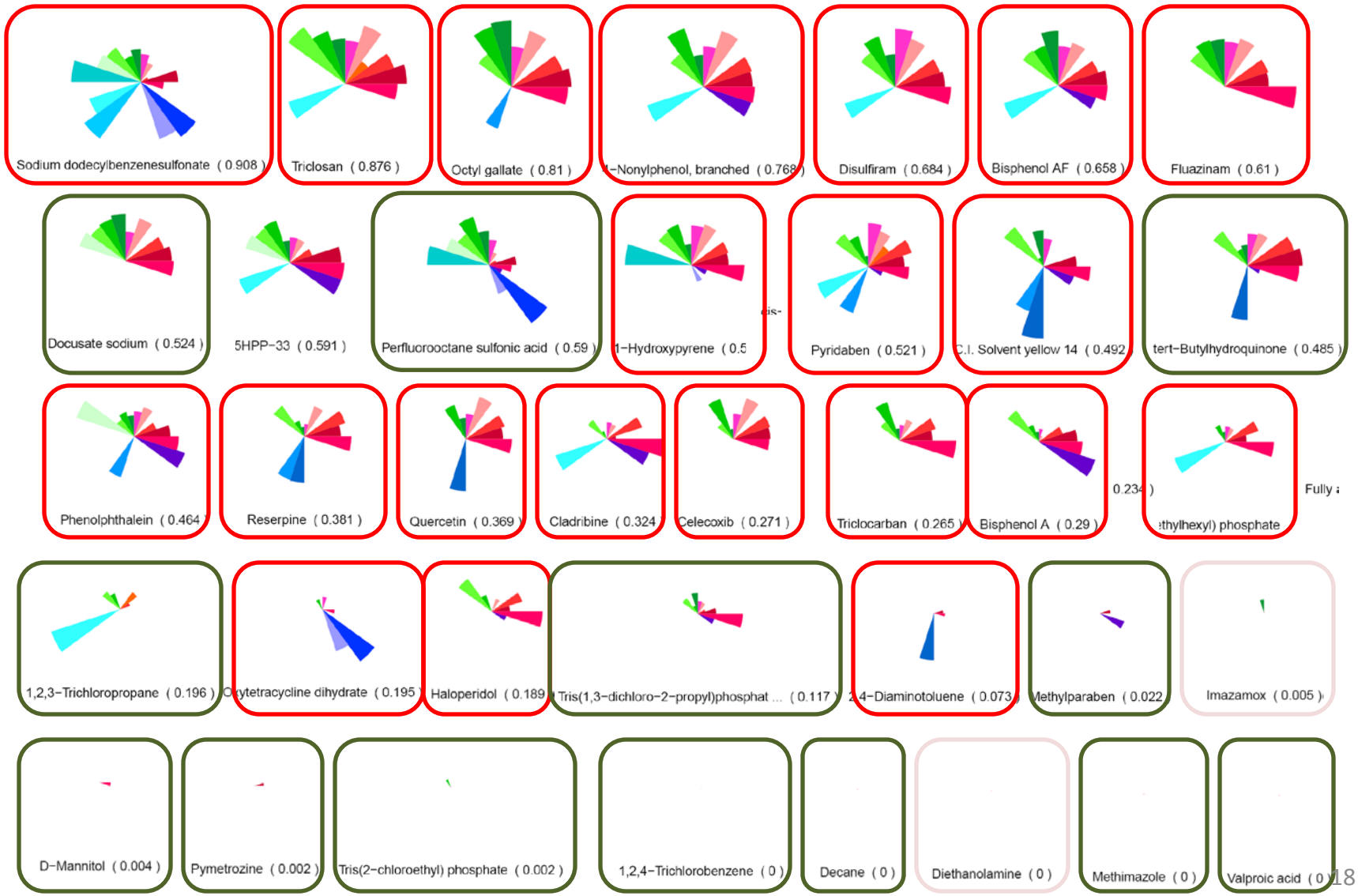
Cranial vascular phenotype: Fluazinam



SOURCE: T. Tal, EPA/NHEERL-ISTD

Vascular Toxicity Results

(Red: hit in at least one assay, Green: no hit)



SUMMARY

- Quantitative AOPs using HTS dose response data allow for hypothesis generation, modeling and testing of MIEs and cellular interactions that may lead to toxicities.

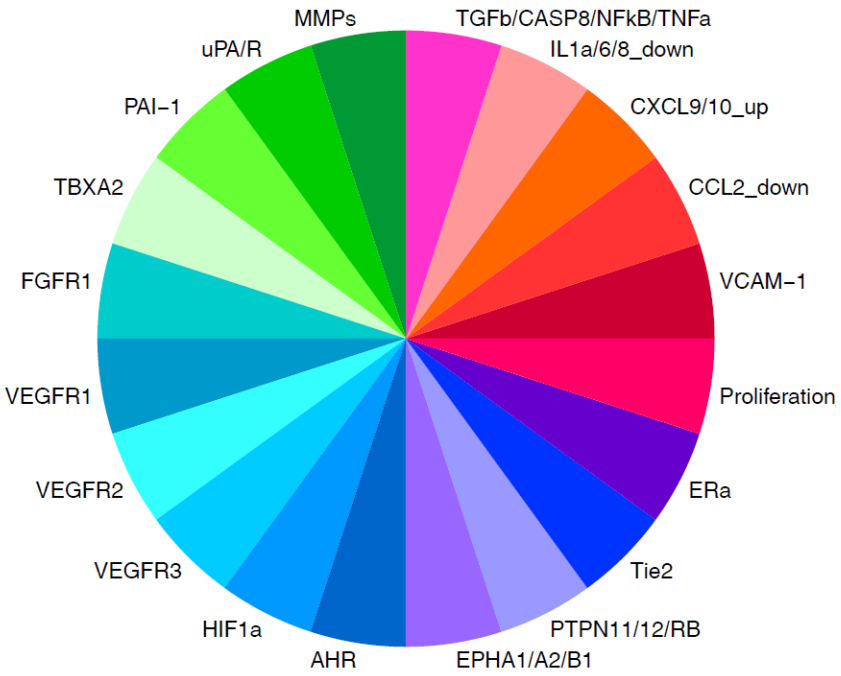
- AOP validation is facilitated via orthogonal assays, small model organisms such as zebrafish, and other scientifically relevant information.

- Our group has generated and evaluated a vascular development screening tool utilizing phenotypic endpoints.

- Preliminary data shows that chemical rankings are generally well correlated among the predictive signature, zebrafish overt toxicity and *in vitro* tubulogenesis assays.

- Validated AOPs enable chemical prioritization, high throughput risk assessments, and probabilistic frameworks.

Future directions: Leveraging diverse data streams to improve the developmental vascular toxicity signature



1. Are all of the assays relevant and how should they be weighted?
2. What non-ToxCast targets are needed?
3. Should other ToxCast assays be included?
4. Are we taking full advantage of the zebrafish model?
5. What other orthogonal assays should be used to test pVDC predictions?

Acknowledgements

Vascular Toxicity Team/ Virtual Embryo Group

- Barbara Abbot (TAD)
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- Raja Settivari (Dow)
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- Charles Wood (ISTD)

FICAM

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- Tuula Heinonen

Padilla Lab

- Shad Mosher
- Alisha Palekar

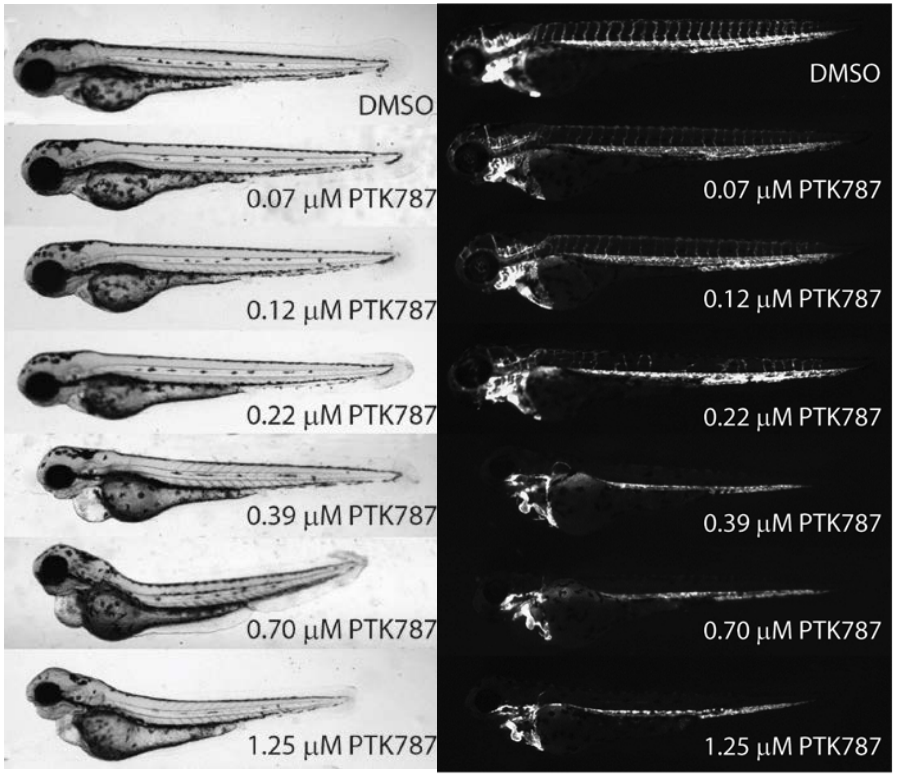
EPA Animal Facility

- Kim Howell
- Ned Collins
- Crystal Walden
- Leslie Martin

Questions?

Functional consequence of vascular disruption during development

A.



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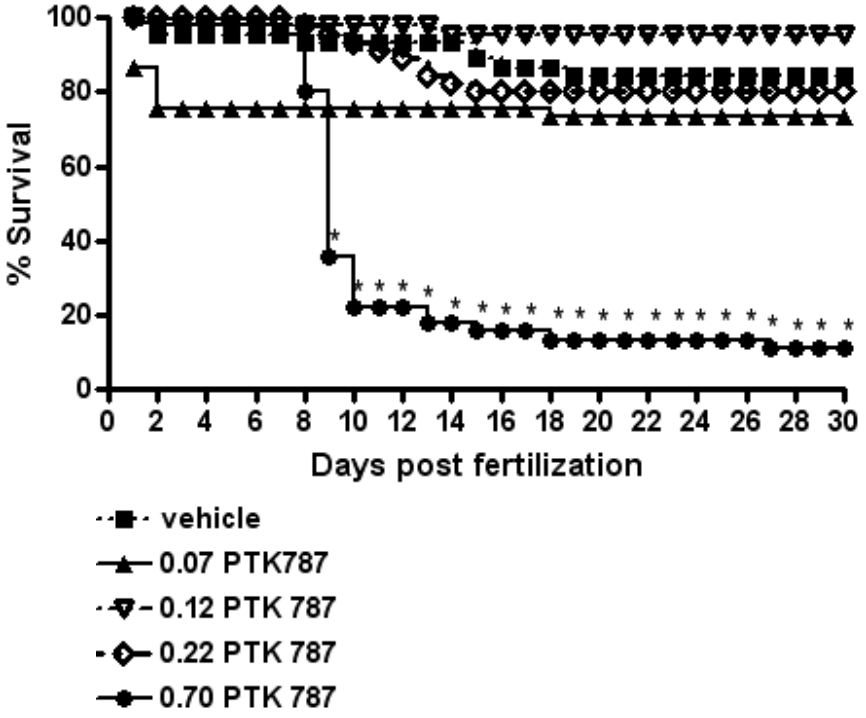

Source: Tal et al. 2014