

# Case Study AOP 43: A Framework for Using Current (and Future) Assays for Predictive DART

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*DART Mechanisms: Strategies to Target In Vitro and In Silico Models to  
Address Data and Assessment Needs*

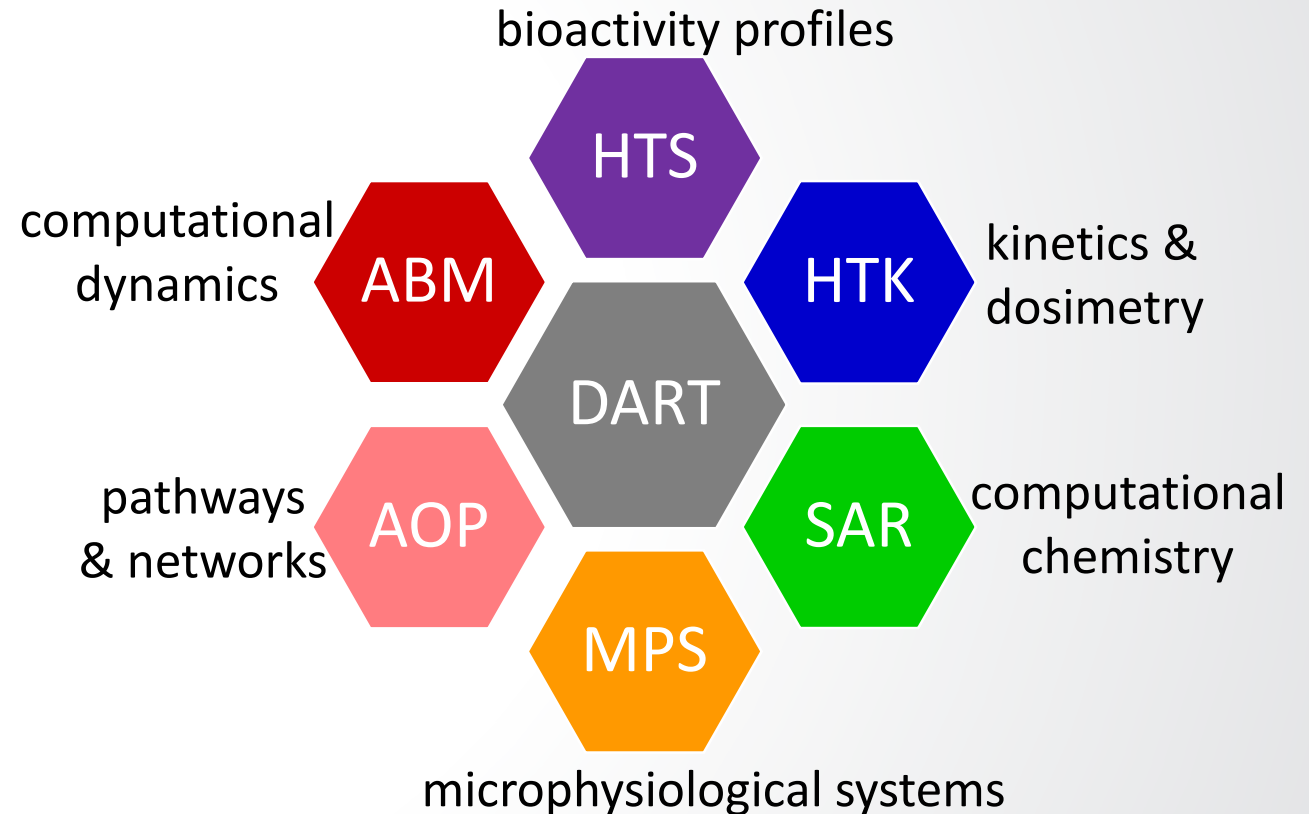
*November 16, 2018*



# Current Testing Paradigm

- Traditional animal-based models for assessing prenatal developmental toxicity (OECD TG 414) expose pregnant rats and/or rabbits during organogenesis and necropsy at term.
- From the animal we get apical endpoints, i.e. skeletal malformation, visceral cleft, growth restrictions, etc.
  - Lack mechanistic depth and detail
  - Does not scale to the human exposure universe

- How can mechanistic information support developmental hazard identification in a 3R's compliant manner?
- How can *in vitro* data and *in silico* models capture the relevant mechanistic information?
- What does this look like within the context of developmental vascular toxicity?



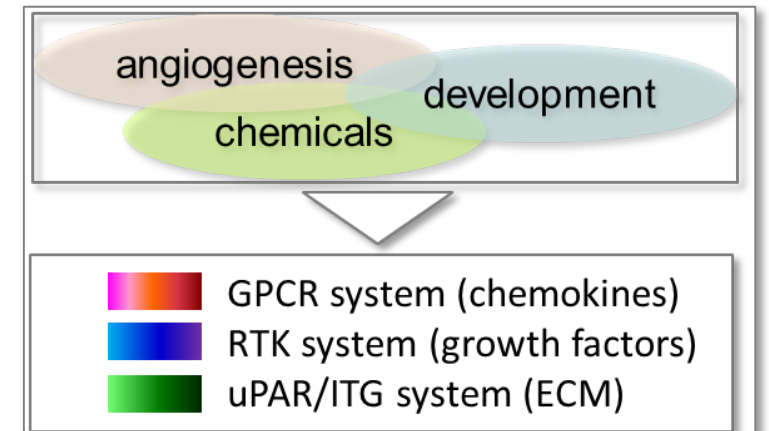


# OECD Aop43: developmental vascular toxicity

## Potential MIE

Inhibition of VEGFR2  
acts disrupts of  
vasculogenesis during  
development following  
several cell and tissue-  
based key events

VDCs



SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res – AOP 43

Chemical structure of 5-hydroxy-2,2',6,6'-tetramethylspiro[3.3]heptan-2-one. The structure features a spirocyclic system with a 5-hydroxyindan-1-one moiety and a 2,2,6,6-tetramethyl-1,2,3,4-tetrahydronaphthalene moiety. The indanone part has a hydroxyl group (HO-) at the 5-position and carbonyl groups (C=O) at the 1 and 3 positions. The naphthalene part has four methyl groups (CH<sub>3</sub>) at the 2, 2', 6, and 6' positions. The spiro center is at the 2-position of the naphthalene ring and the 1-position of the indanone ring.

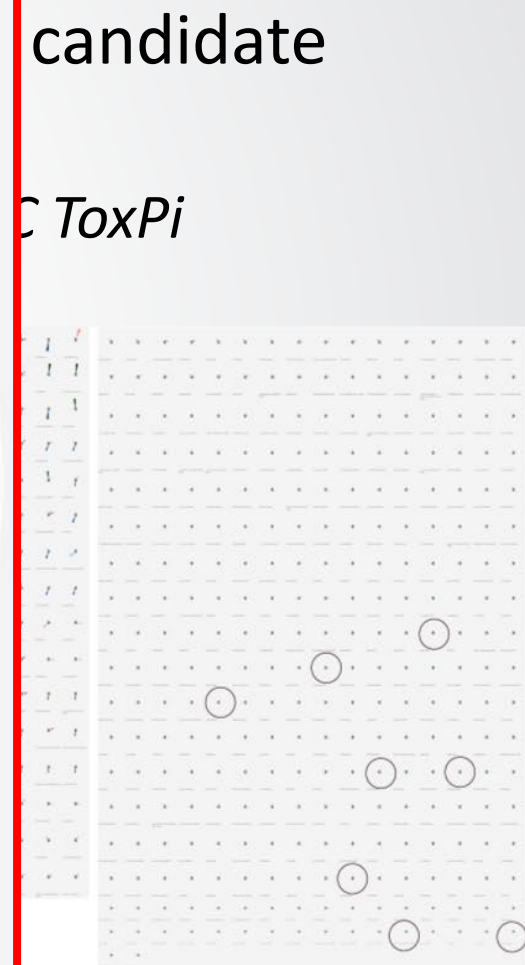
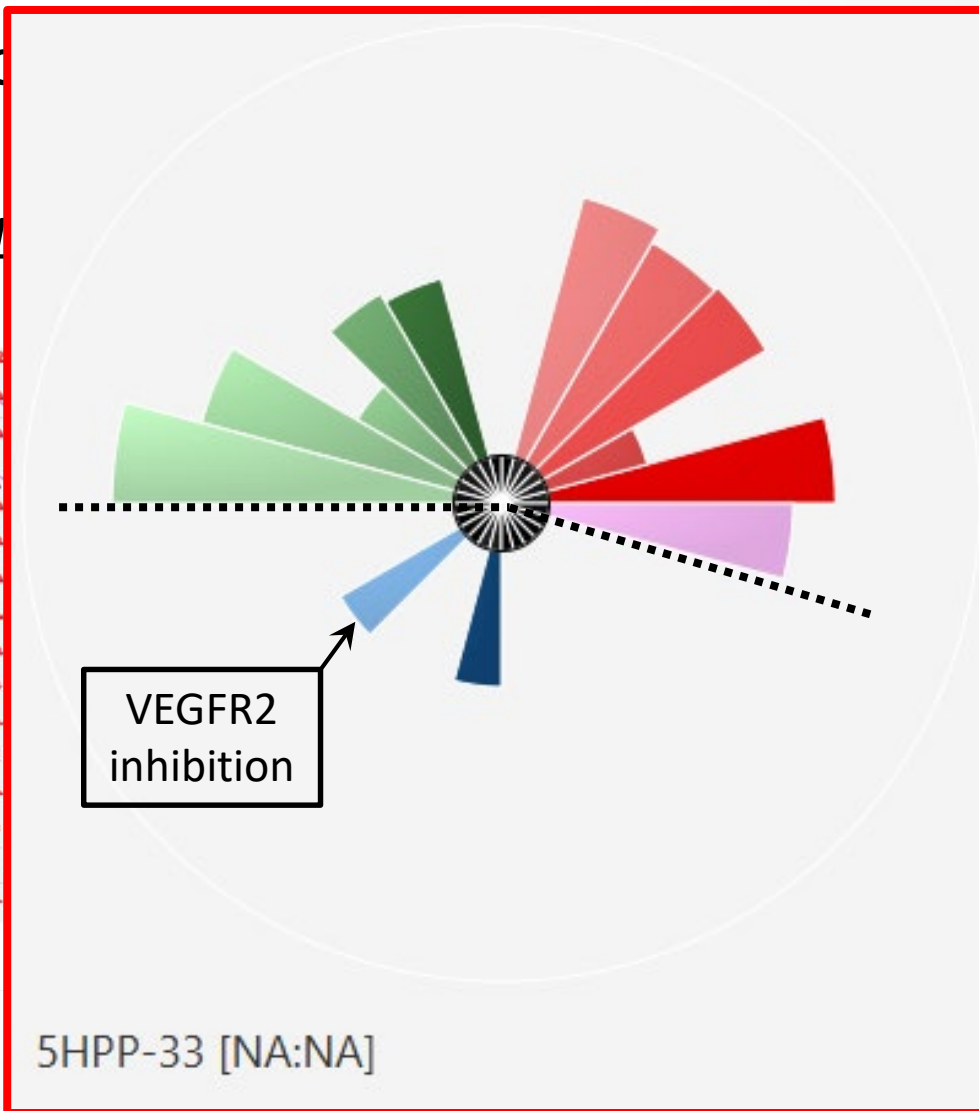
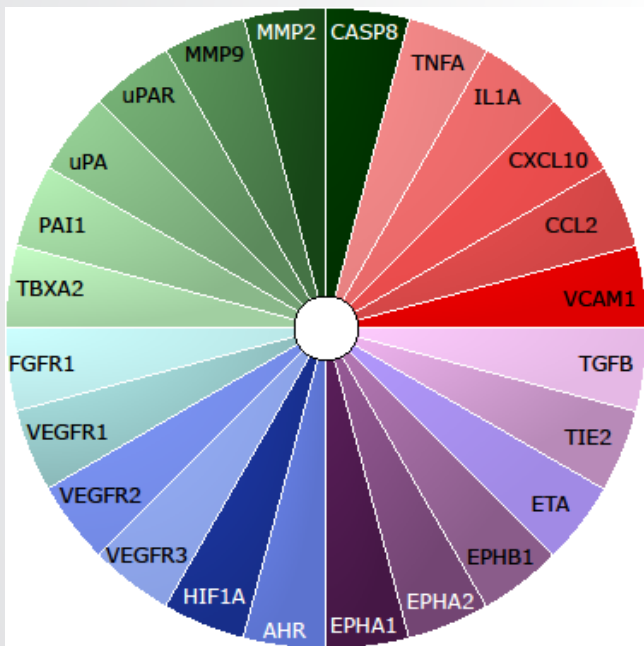




# Molecular Initiating Event (MIE)

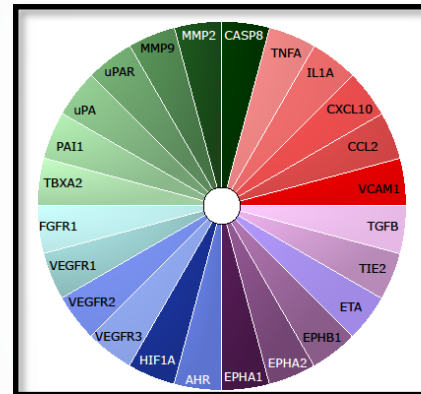
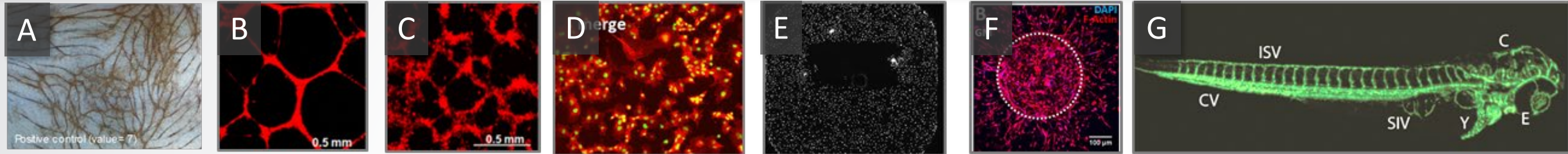
- Utilize high throughput screening to identify candidate

24 ToxCast target assays  
(pVDC ToxPi)



SOURCE: Kate Saili, NCCT

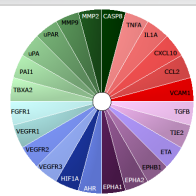
# Validating angiogenic cycle key events



*Utilize cell-based assays across multiple angiogenic effects to quantitate cellular key events*

- A. Tubule formation and network formation
- B. Tubulogenesis in high throughput screening
- C. Endothelial network formation in co-culture
- D. nuCTNB and endothelial migration
- E. Endothelial cell migration assay
- F. 3D angiogenic sprouting
- G. KDR-reporter transgenic zebrafish embryos

# *In vitro* confirmation of *in silico* prediction



	A	B	C	D	E	F	G	H	I	J	K	L
	ToxCast pVDC	FICAM	tubulogenesis NCATS	tubulogenesis synthetic	tubulogenesis Matrigel	tubulogenesis nuCTNB	EC Migration	Sprouting UWisc	ZF-TG embryo	ZF hyaloid	VALA	ANY
Decane	0											0
1,2,3-Trichloropropane	0											0
Pymetrozine	0											0
Methimazole	0											0
Imazamox	0											0
D-Mannitol	0											0
Methylparaben	0											0
Valproic acid	0											0
Tris(2-ethylhexyl) phosphate	0											0
TNP-470	1											1
4-Nonylphenol, branched	1											1
1,2,4-Trichlorobenzene	2											2
Diethanolamine	2											2
Reserpine	2											2
Sodium dodecylbenzenesulfonate	2											2
Oxytetracycline dihydrate	2											2
Quercetin	2											2
Tris(2-chloroethyl) phosphate	3											3
2,4-Diaminotoluene	3											3
Tris(1,3-dichloro-2-propyl)phosphate	3											3
Celecoxib	3											3
C.I. Solvent Yellow 14	3											3
tert-Butylhydroquinone	4											4
Triclosan	4											4
Bisphenol AF	4											4
Haloperidol	4											4
Docusate sodium	5											5
Cladribine	5											5
Triclocarban	5											5
Pyridaben	5											5
1-Hydroxypyrene	5											5
Disulfiram	5											5
Bisphenol A	5											5
Fluazinam	6											6
Phenolphthalein	6											6
Octyl gallate	6											6
5HPP-33	8											8

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)

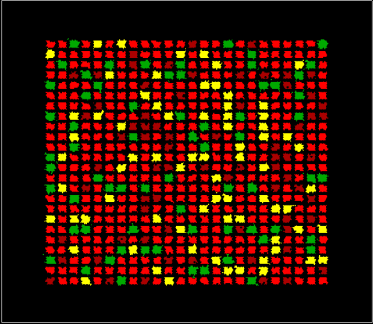
J reporter zebrafish (UDUBLIN)

K HUVEC tubulogenesis (VALA)

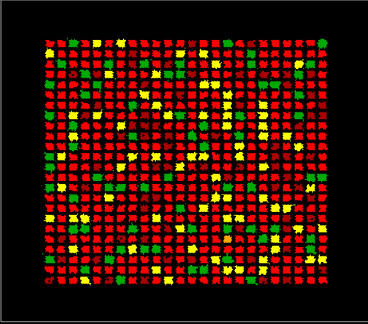
L ANY (B to K)

# Tissue-level key events and embryotoxicity

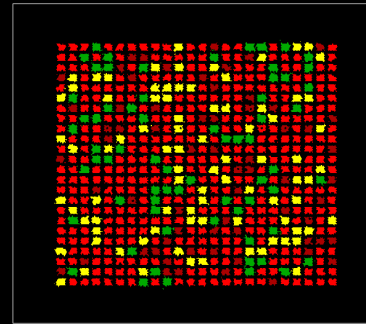
control



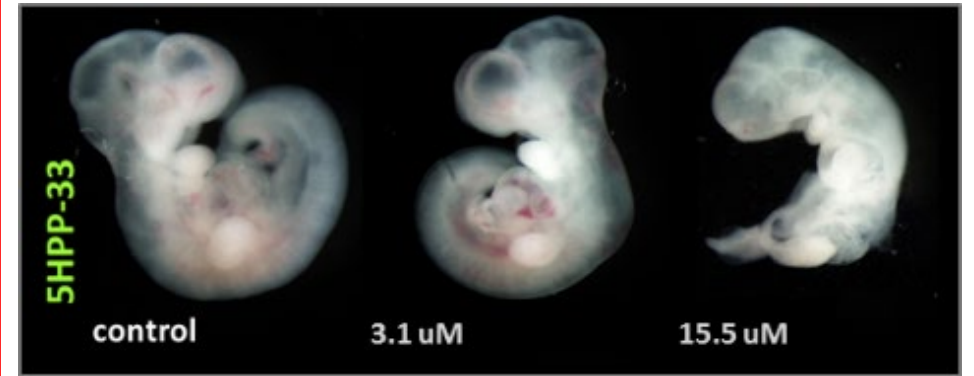
5  $\mu\text{M}$



40  $\mu\text{M}$

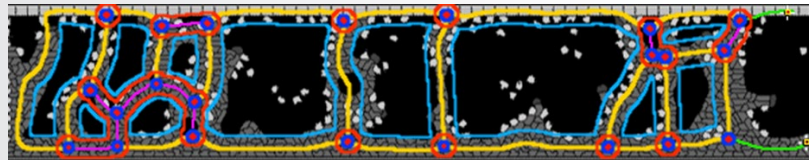


SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol 9(4): e1002996



- $\downarrow$  endothelial networks
- critical effect - embryo viability
- AC50 = 21.2  $\mu\text{M}$

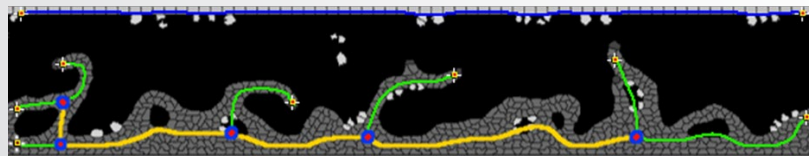
SOURCE: Ellis-Hutchings et al. (2017) Reprod Toxicol



0.03  $\mu\text{M}$

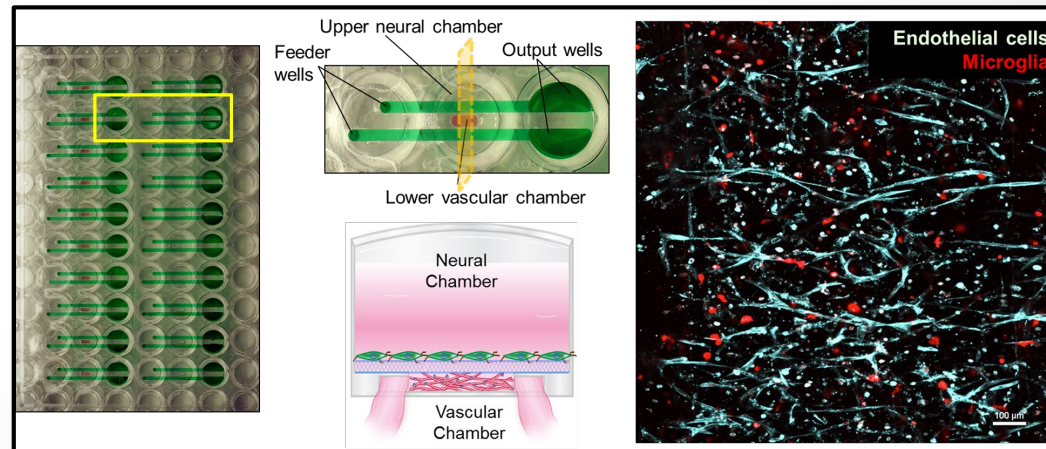


0.3  $\mu\text{M}$



6.0  $\mu\text{M}$

Todd Zurlinden - NCCT

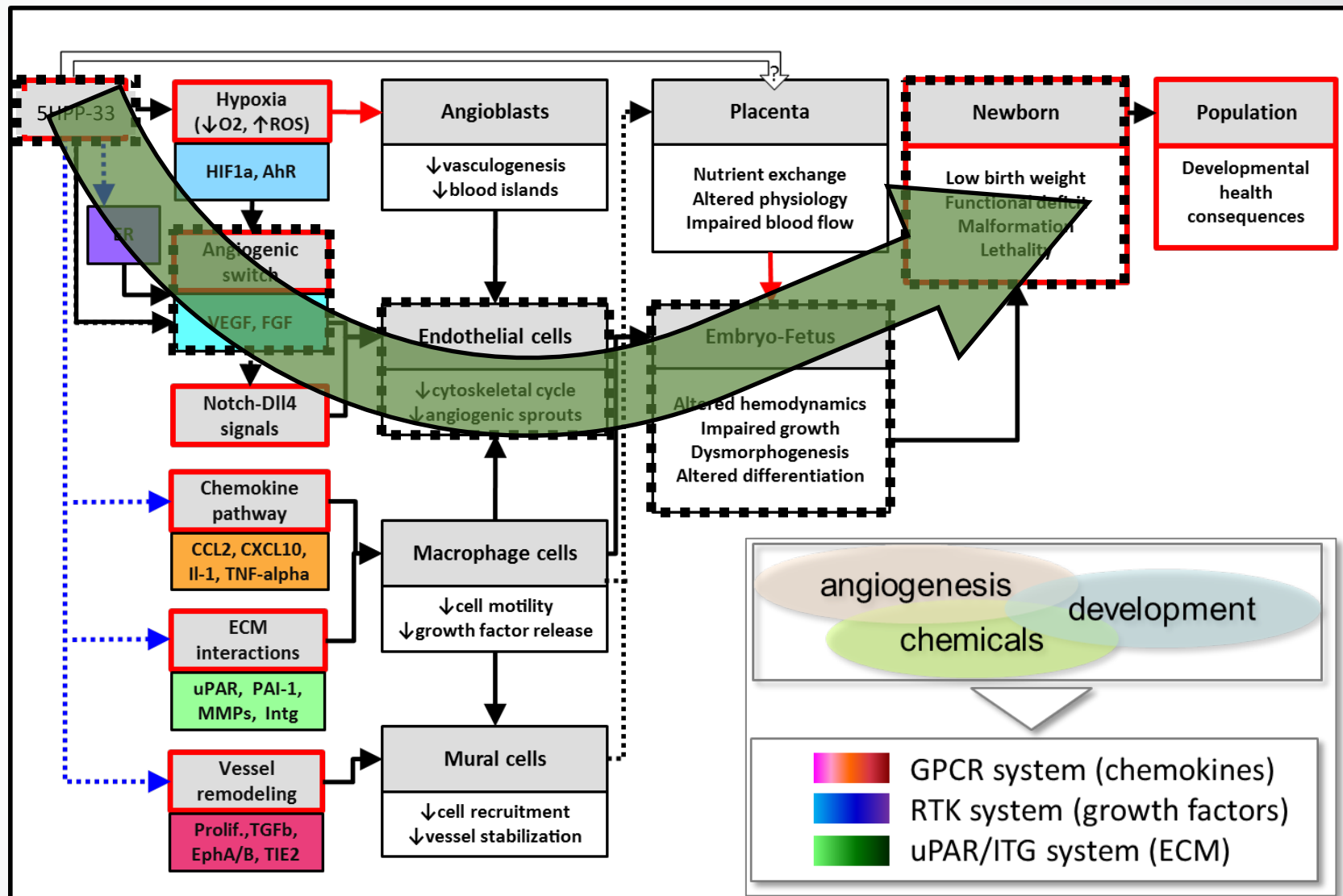
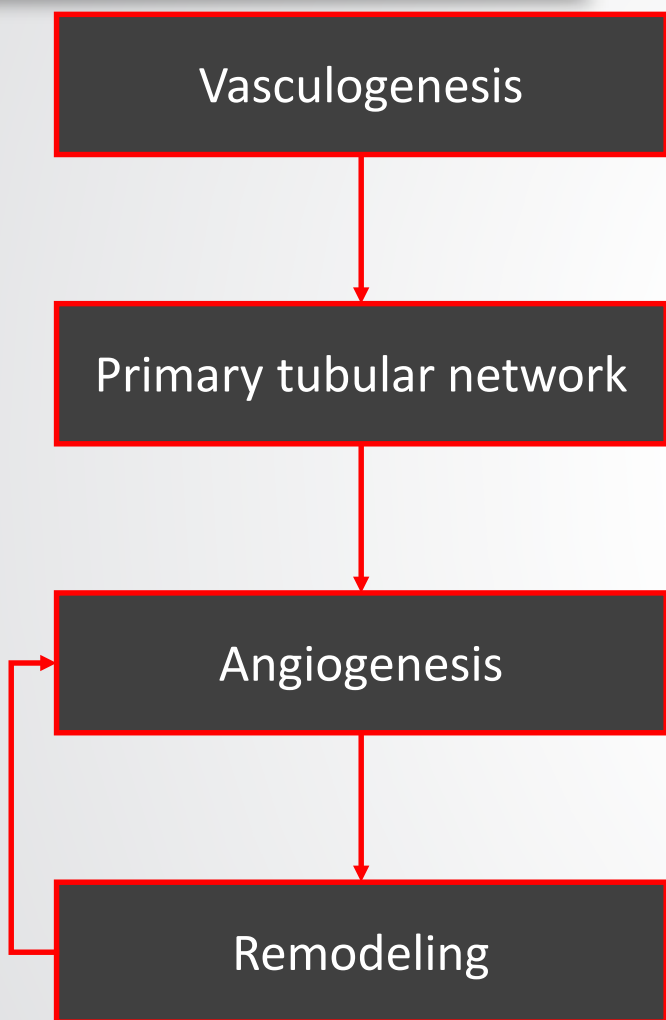


**Biomimetic  
reconstruction  
(hNVU)**

W Murphy, W Daly, G Kaushick – U Wisconsin (HMAPS)



# Organism Adverse Outcome

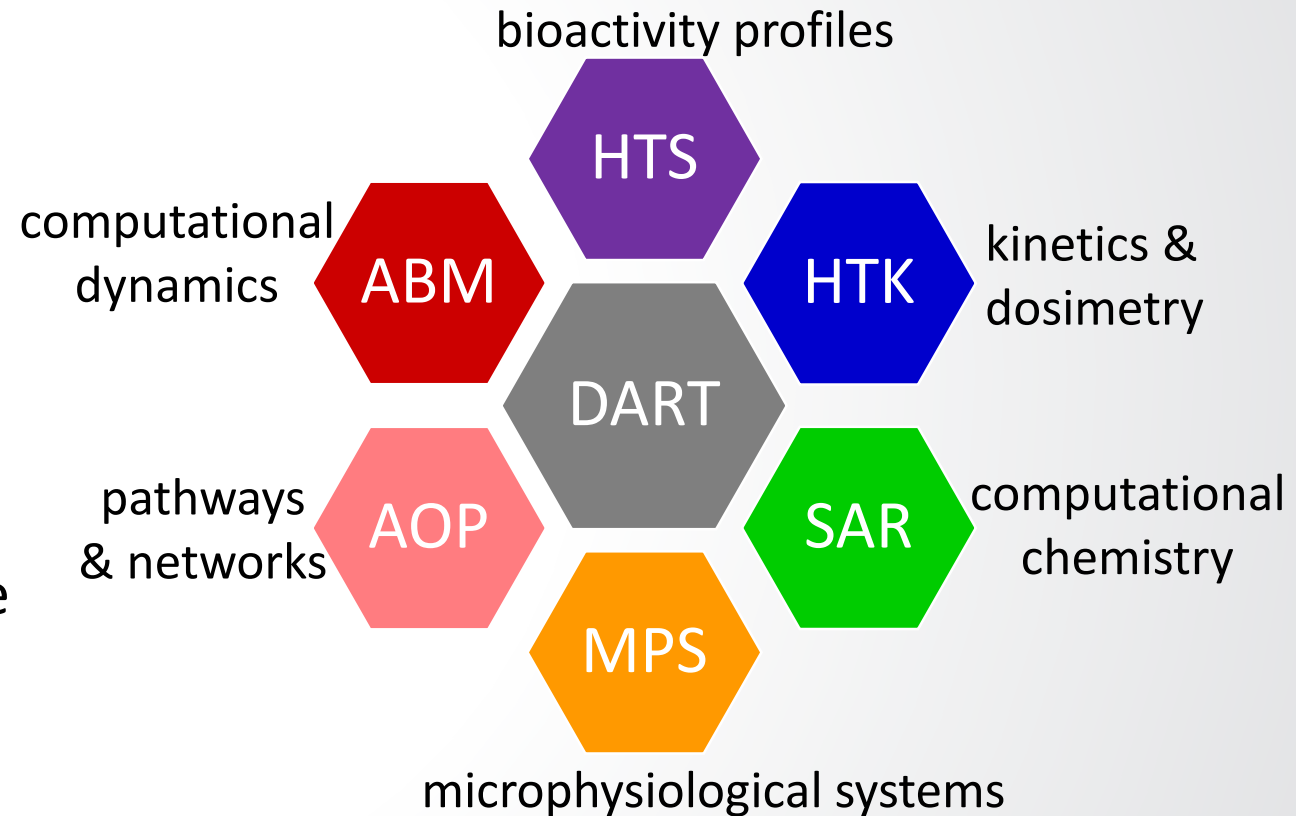


SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res – AOP 43



# Current (and future) assays

- Establish suite of gene targets from the literature potentially resulting in angiogenic disruption
  - Organize as MIEs within context of AOP network
- Use ToxCast HTS assays to determine how a chemical enters the AOP
- Validate cellular-level effect through suite of assays corresponding to different parts of the angiogenic cycle
- Predict system dynamics for test compound and similar compounds using HTS data and OCM and agent-based modes for cell-cell interactions





# Thank You

Questions?