Microcephaly

computational and organotypic modeling of a complex human birth defect

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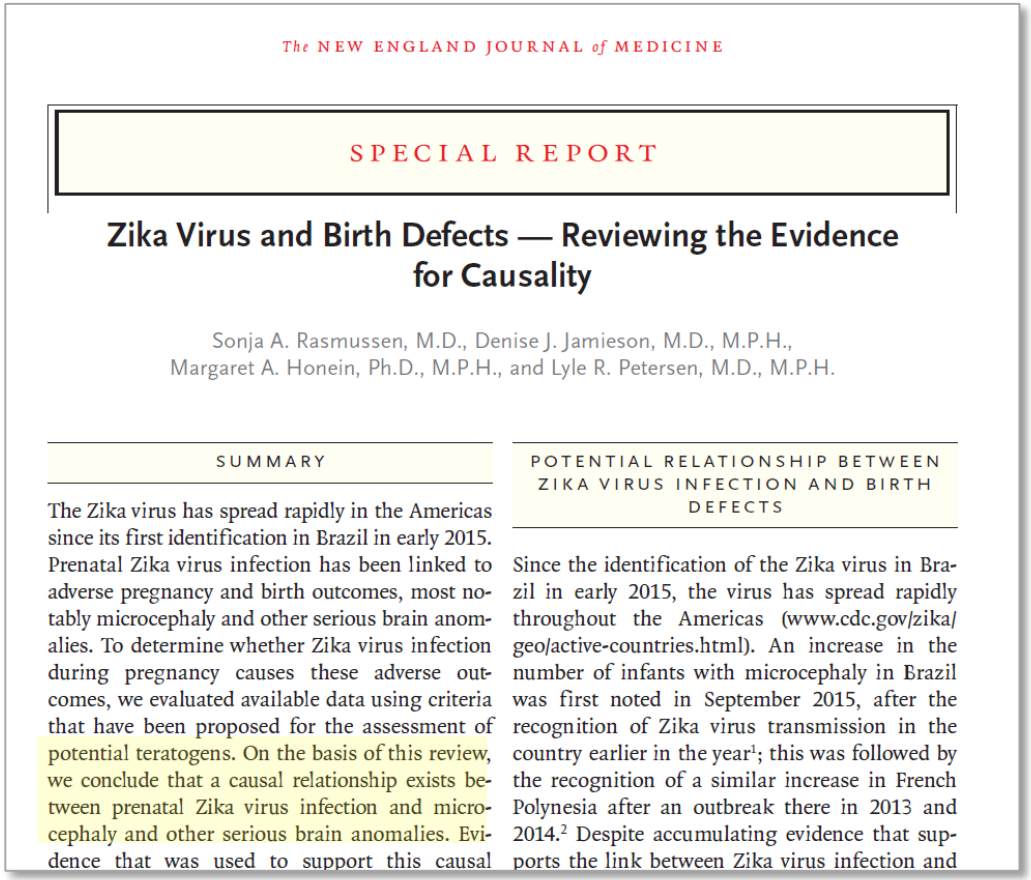
Zika virus and the recent outbreak of Microcephaly

Vogel (2016) Science 351

- Cluster of severe birth defects first noted in Northern Brazil, September 2015, correlating with an outbreak of the Zika virus (ZIKV) starting in April 2015 [1].
- ZIKV is spread primarily by the *Aedes aegypti* mosquito, which also transmits dengue, yellow fever and chikungunya (*Flaviviridae* order of RNA viruses).
- No flavivirus known to cause human birth defects; in an unprecedented move the Brazilian Health Ministry linked the epidemic to ZIKV (December, 2015).
- CDC tested 4 microcephalic births and found positive evidence of ZIKV genetic material with same sequence as maternal ZIKV rash during pregnancy [2].

Etiology Timeline 2016

- WHO declared the ZIKV epidemic a Public Health Emergency of International Concern (PHEIC) (February 2016) mobilizing a task force [3].
- Zika Embryopathy Task Force Registry connected microcephaly to maternal rash during the 1st trimester (57% cases) and 2nd trimester (14% cases).
- Local physicians in Brazil suggested mass-spraying by the mosquito larvicide 'pyroproxyfen' (JH antagonist) used in drinking water was the real culprit.
- EPA and WHO find no evidence for prenatal developmental toxicity for the larvicide beyond what could be attributed to maternal toxicity in rats/rabbits.
- Surge of information connects ZIKV with microcephaly: "*Now there's almost no doubt that Zika is the cause*." - AS Fauci, NIH/NIAID
- February 19: NIH issued an RFA for exploratory research on "Rapid Assessment of Zika Virus (ZIKV) Complications" (PAR-16-106).



The NEW ENGLAND JOURNAL of MEDICINE

SPECIAL REPORT

Zika Virus and Birth Defects — Reviewing the Evidence for Causality

Sonja A. Rasmussen, M.D., Denise J. Jamieson, M.D., M.P.H., Margaret A. Honein, Ph.D., M.P.H., and Lyle R. Petersen, M.D., M.P.H.

SUMMARY

The Zika virus has spread rapidly in the Americas since its first identification in Brazil in early 2015. Prenatal Zika virus infection has been linked to adverse pregnancy and birth outcomes, most notably microcephaly and other serious brain anomalies. To determine whether Zika virus infection during pregnancy causes these adverse outcomes, we evaluated available data using criteria that have been proposed for the assessment of potential teratogens. On the basis of this review, we conclude that a causal relationship exists between prenatal Zika virus infection and microcephaly and other serious brain anomalies. Evidence that was used to support this causal

POTENTIAL RELATIONSHIP BETWEEN ZIKA VIRUS INFECTION AND BIRTH DEFECTS

Since the identification of the Zika virus in Brazil in early 2015, the virus has spread rapidly throughout the Americas (www.cdc.gov/zika/geo/active-countries.html). An increase in the number of infants with microcephaly in Brazil was first noted in September 2015, after the recognition of Zika virus transmission in the country earlier in the year[1]; this was followed by the recognition of a similar increase in French Polynesia after an outbreak there in 2013 and 2014.[2] Despite accumulating evidence that supports the link between Zika virus infection and

"On the basis of this review, we conclude that a causal relationship exists between prenatal Zika virus infection and microcephaly and other serious brain anomalies."

N ENGL J MED, April 14, 2016 [4]

No 'smoking gun' but weight of evidence supports causality:

- ZIKV infection times in pregnancy consistent with defects observed (critical windows);
- presence of ZIKV genetic material in brain tissue of affected infants (evidence for exposure);

ZIKV disrupts neuroprogenitor cell growth in human iPSC models (biologic plausibility).

Zika: EPA Outline of Plan for the United States and the Pacific Islands

2/10/2016 draft

"As part of our mission to protect human health and safeguard the environment, EPA is working with our federal partners and other stakeholders to combat the recent outbreak of the Zika virus. Because the Zika virus is vectored by mosquitoes (Aedes aegypti and Aedes albopictus), EPA's role is to encourage responsible and effective mosquito control, including Integrated Pest Management (IPM), and individual protection from mosquito bites".

For example:

1. Protection from mosquito bites, use of indoor spray products, mosquito control;
2. Education, outreach and training on safe use of repellent products;
3. Collaboration with CDC and other agencies on scientific and technical support to assess and manage potential impacts to human populations.

Need for Computational Models:
problem formulation scales to a broader framework

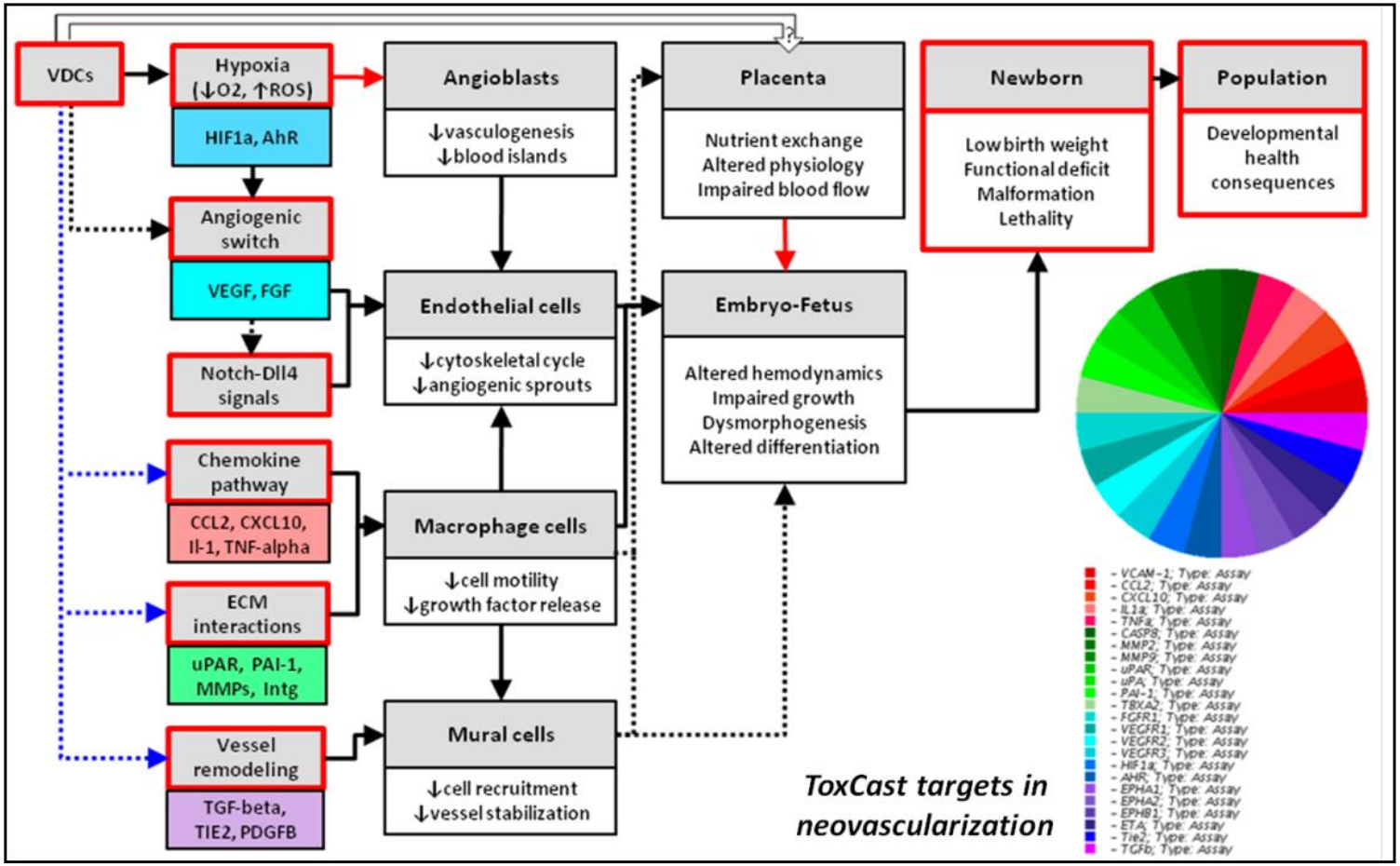
- Microcephaly may be the 'tip of the' iceberg and many conditions are known to evoke this adverse outcome during early pregnancy [5]:
    - intrauterine infections (e.g., Rubella, CMV, ZikV)
    - inborn errors of metabolism (e.g., urea cycle, mitochondriopathies)
    - maternal smoking, drug and alcohol abuse
    - fetal hypoxia, perinatal brain damage
    - environmental chemicals (e.g., methylmercury)
    - genetic factors (autosomal recessive traits; microdeletions, duplications)
    - prenatal malnutrition, socioeconomic factors.
- Need arises for computational and experimental models that can be used to probe and validate the many possible explanations and connections.
- Case use for an Adverse Outcome Pathway (AOP) framework of 'microcephaly' that scales to ToxCast/Tox21 libraries for predictive toxicology.

Predictive Toxicology & Human Development

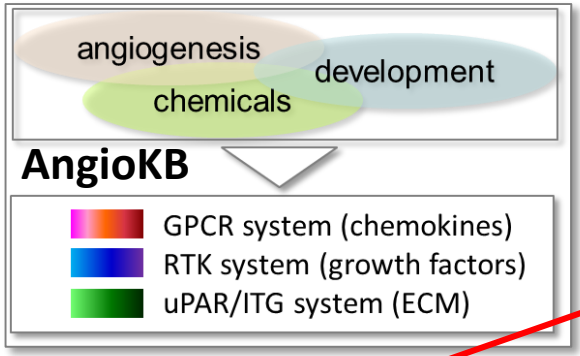
- Evaluating and assessing impacts to development is an Agency priority – *EPA's Children's Environmental Health (CEH) Research Roadmap.*
- Too many chemicals (~80K) to test each by traditional animal-based methods (cost, time, 3Rs).
- Profile the 'human exposure universe' of chemicals *in vitro* with high-throughput (HTS) assays (ToxCast/Tox21).
- ToxCast >1060 chemicals evaluated in over 600 assays; >27M data points and ~1.7M concentration response curves.

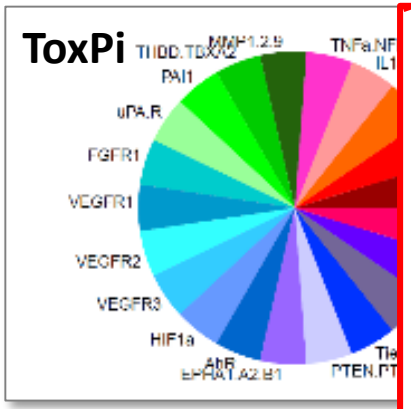
http://actor.epa.gov/dashboard/

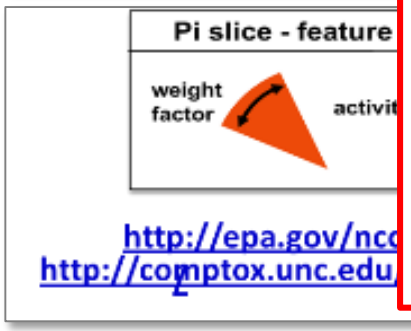
EXAMPLE: AOP framework for embryonic vascular disruption [6]

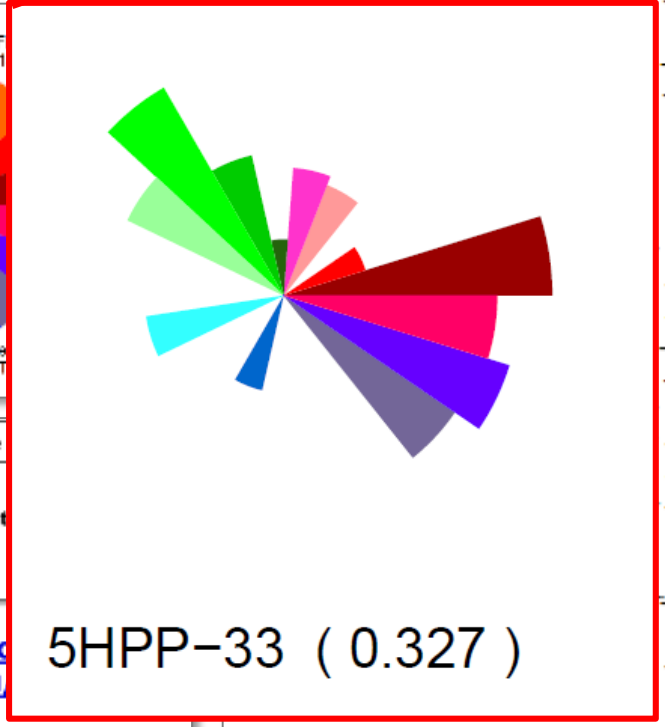


ToxCast chemicals: potential for vascular disrupton (pVDCs)







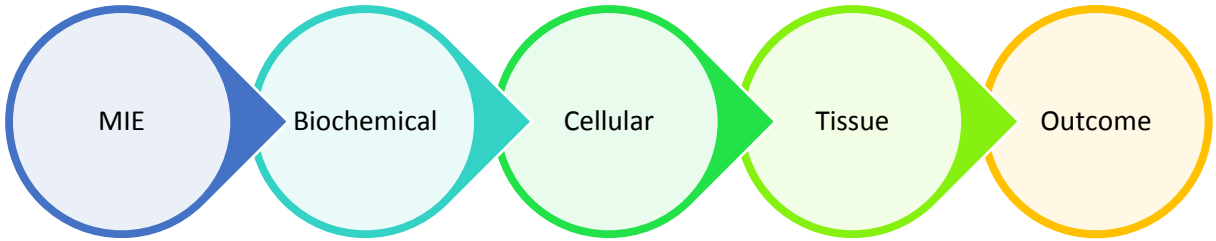


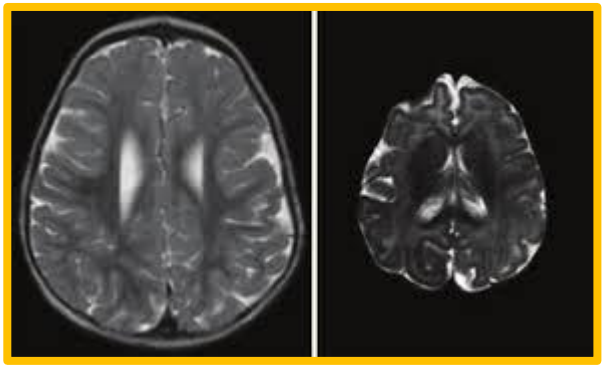
AOP-based prediction model is 85% accurate in discriminating pVDCs from non-pVDCs in an OECD-validated human complex angiogenesis assay.

FICAM Finnish Centre for Alternative Methods

T Heinonen

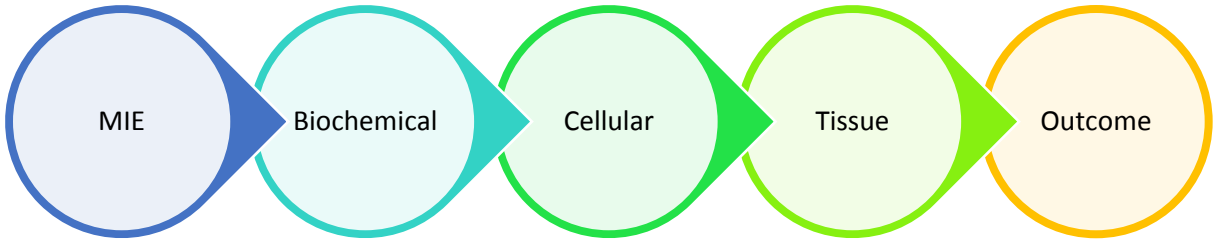
This synthetic thalidomide analogue disrupts microtubule function in endothelial cells of immature blood vessels.

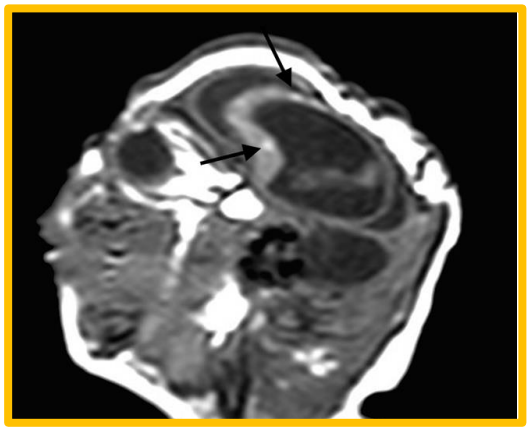




Adverse Outcome: *microcephaly*

- Craniofacial disproportion is not straightforward to diagnose; a standardized diagnostic approach has been devised [5].
- Reduction in head circumference relative 3 STDEV below the population mean by age and gender.
- Prenatal imaging picks up the reduction in brain volume with ventricular dilations by the 3rd trimester (harder to pick up earlier).
- Other defects include brain calcifications, retinal/optic nerve defects, and shrunken placenta.

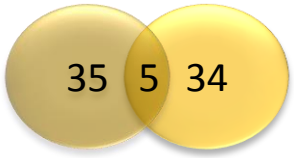




Aragao et al. (2016) [7]

Adverse Outcome: *microcephaly*

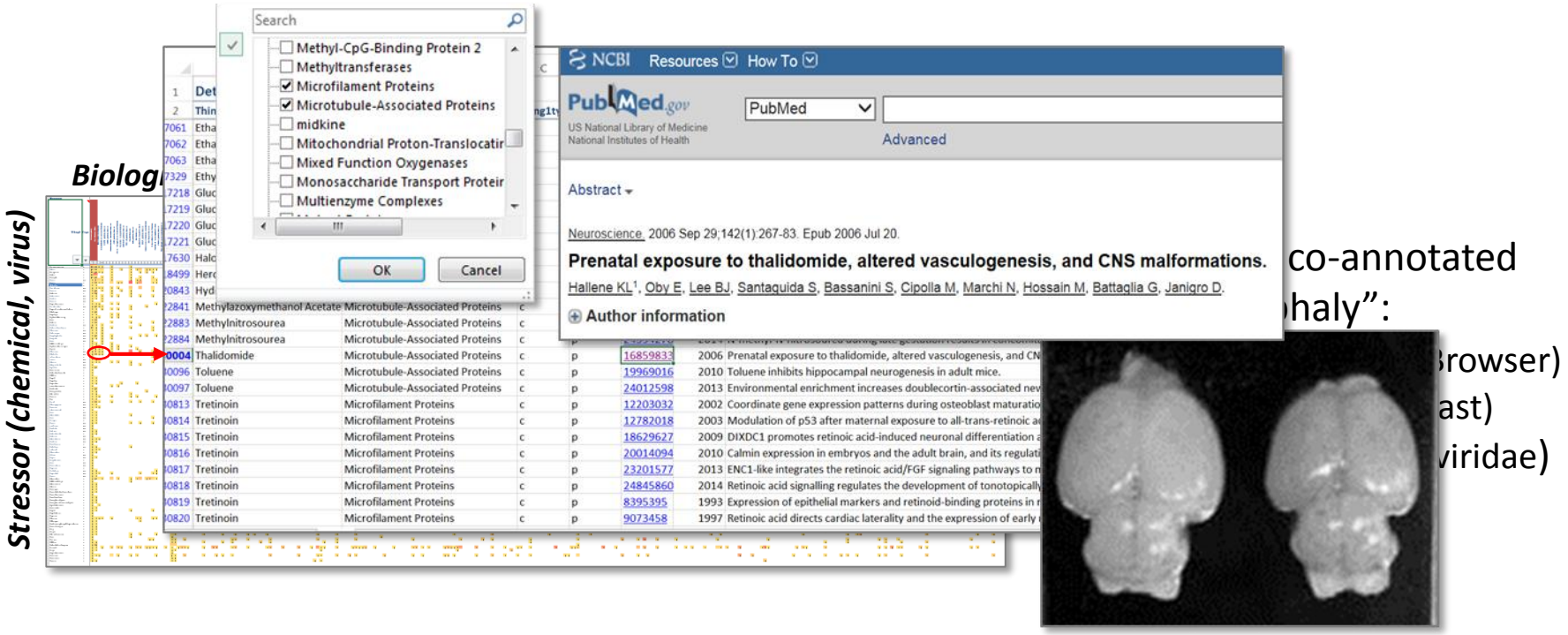
- In primary microcephaly, brain growth starts out normally (e.g., all major parts form in the embryo) but then slows or atrophies during the 2nd trimester:
  - reduction in cortical surface area and ventricular dilations (resolving as hydrocephalus due to impairment of CSF flow) are the main features as with other viral teratogens [7].
- Mammalian Phenotype Browser: 'microcephaly' (MP:0000433) returns 85 gene associations including candidate genes for primary microcephaly in humans.
- ToxRefDB shows 75 chemicals with relevant, nonsystemic developmental effects:

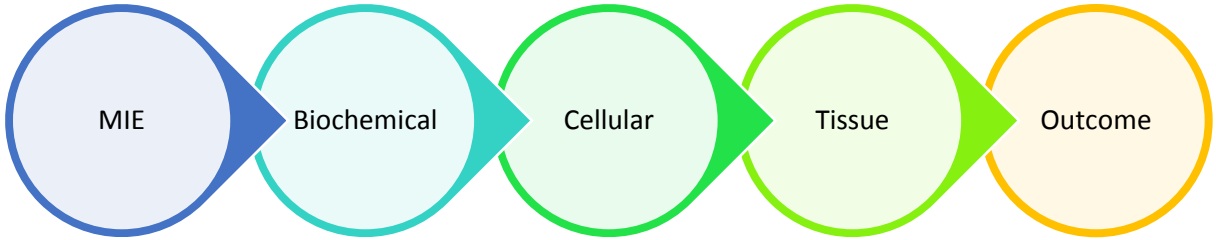


  - 40 (51%) → reductions in brain size or cellular mass
  - 39 (52%) → dilated ventricles or hydrocephaly
  - 5 (6.3%) → both types of effects.

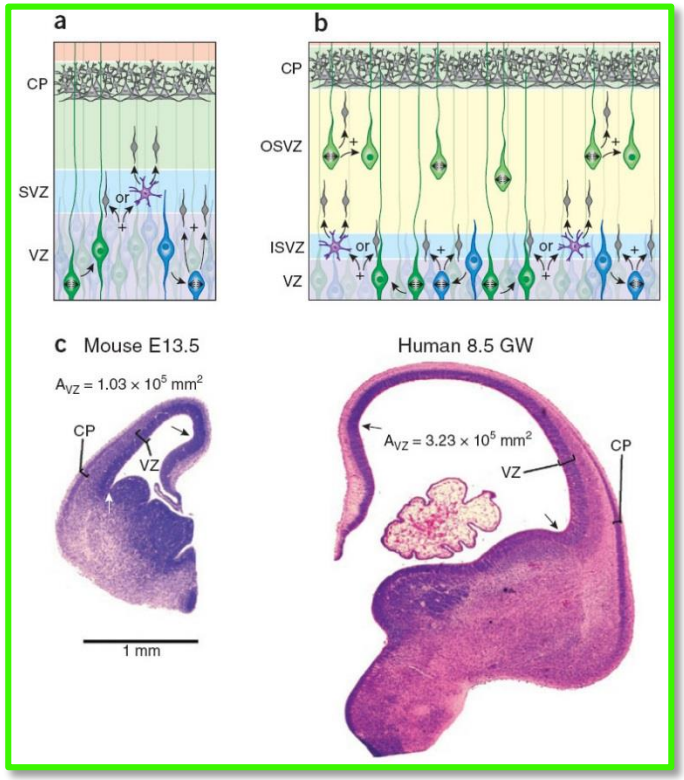
Knowledge Framework

- *MicrocephalyConnections* tool sweeps literature (PubMed) for references relevant to gene-, chemical-, or viral- effects on development [N Baker, NCCT].



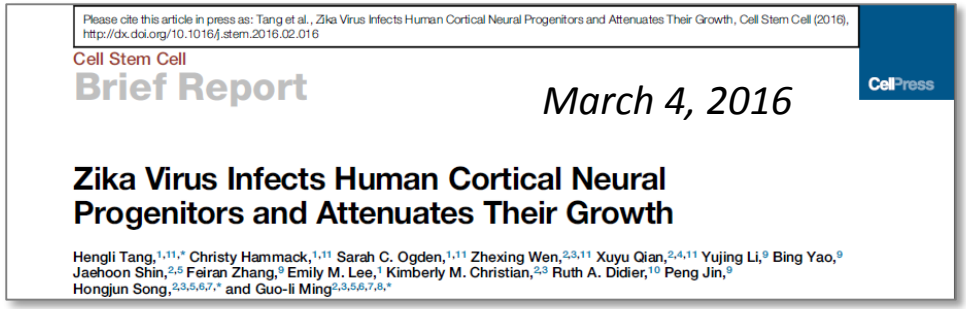


Target Tissue: *developing ventricular zone*



Cortical lamination in the embryo [8]

- Neuroprogenitor cells (NPCs) self-replicate in the ventricular zone during 1st trimester.
- Growth period is followed by differentiation to form neocortex by the second trimester.
- Logistical dynamics of NPC growth is key factor in setting brain size during corticogenesis [9].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Cell Stem Cell

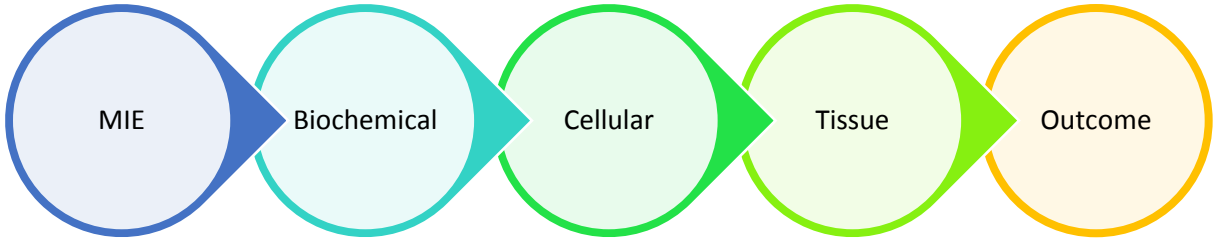
Brief Report

March 4, 2016

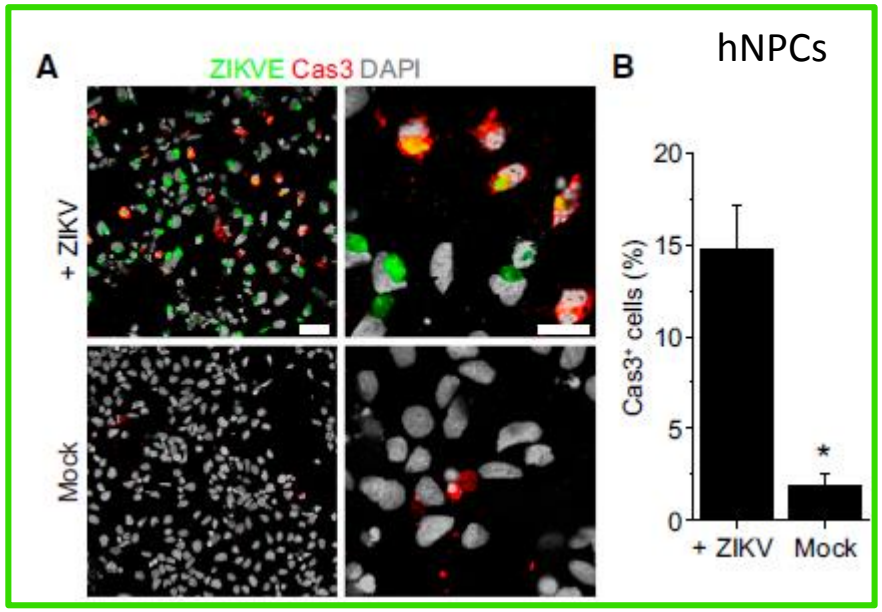
CellPress

Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth

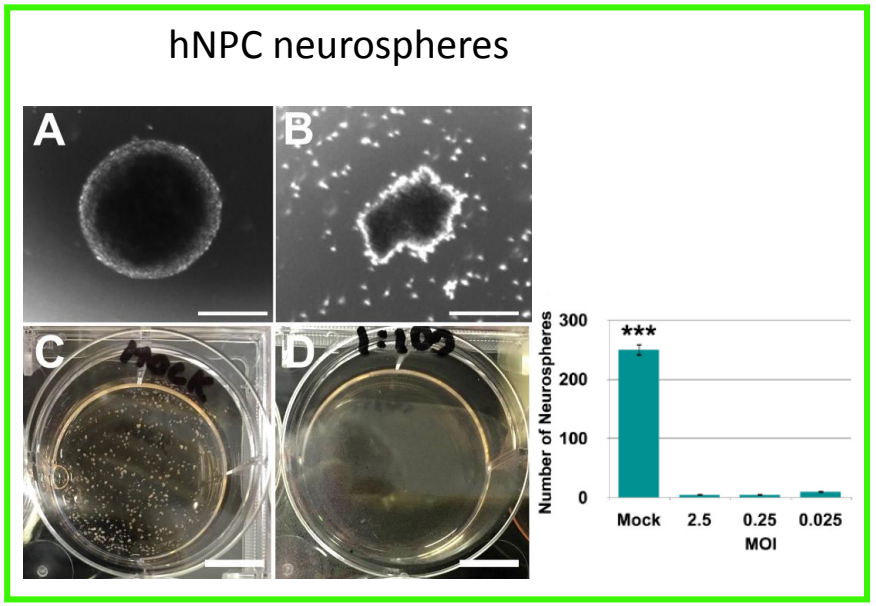
Hengli Tang,[1,11,*] Christy Hammack,[1,11] Sarah C. Ogden,[1,11] Zhexing Wen,[2,3,11] Xuyu Qian,[2,4,11] Yujing Li,[9] Bing Yao,[9] Jaehoon Shin,[2,5] Feiran Zhang,[9] Emily M. Lee,[1] Kimberly M. Christian,[2,3] Ruth A. Didier,[10] Peng Jin,[9] Hongjun Song,[2,3,5,6,7,*] and Guo-li Ming[2,3,5,6,7,8,*]



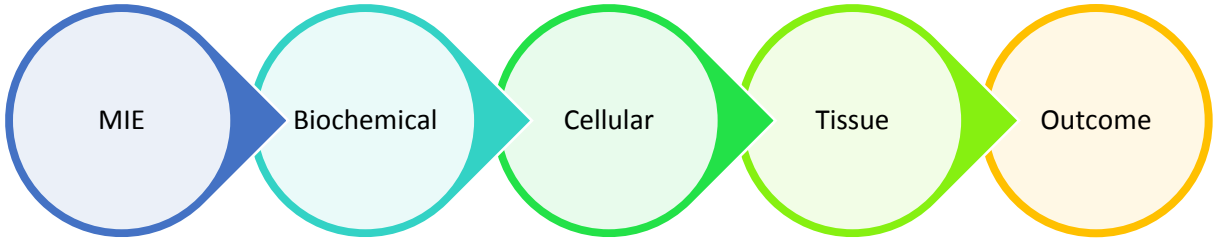
Key Event: *Reduced neuroprogenitor cell (NPCs) population size*.



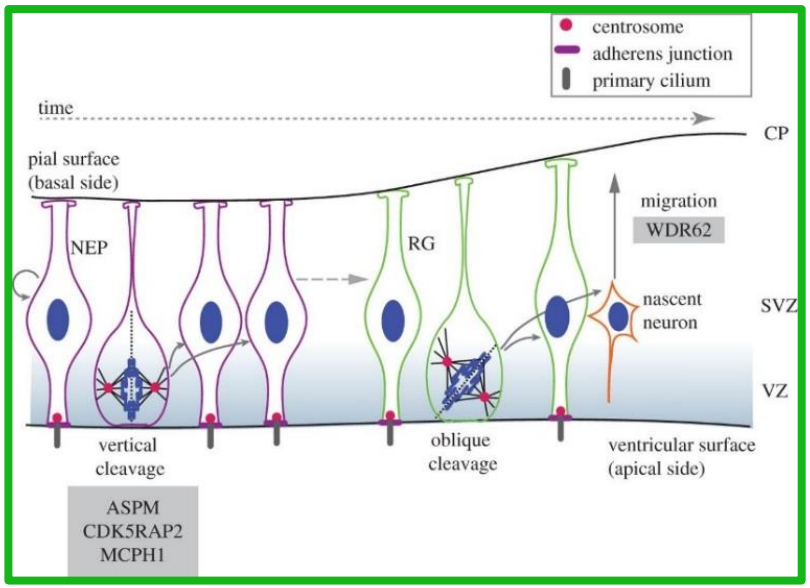
Tang et al. (2016) [10]



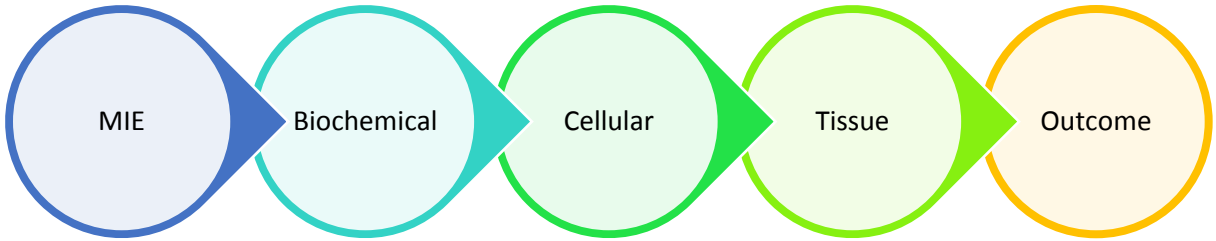
Garcez et al. (2016) [11]

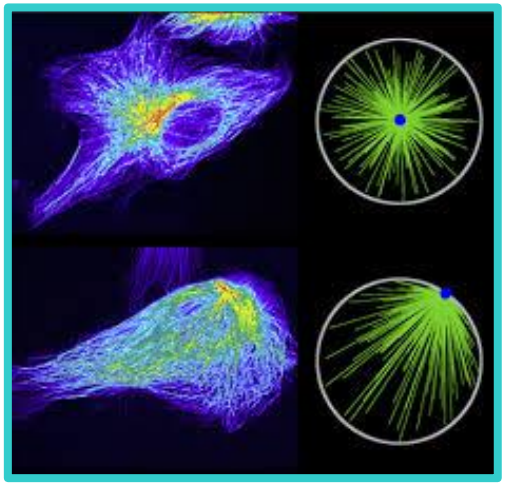


Cellular Event: *orientation of the mitotic plane*



- NPCs divide symmetrically before switching to asymmetric (neurogenic) divisions.
- Premature switching (or apoptosis) results in loss of NPCs from the proliferative cycle.
- This ultimately results in a reduction of neurons and in turn a smaller brain.
- Positioning of the mitotic spindle is a key determinant of NPC pool size at the onset of neurogenesis.

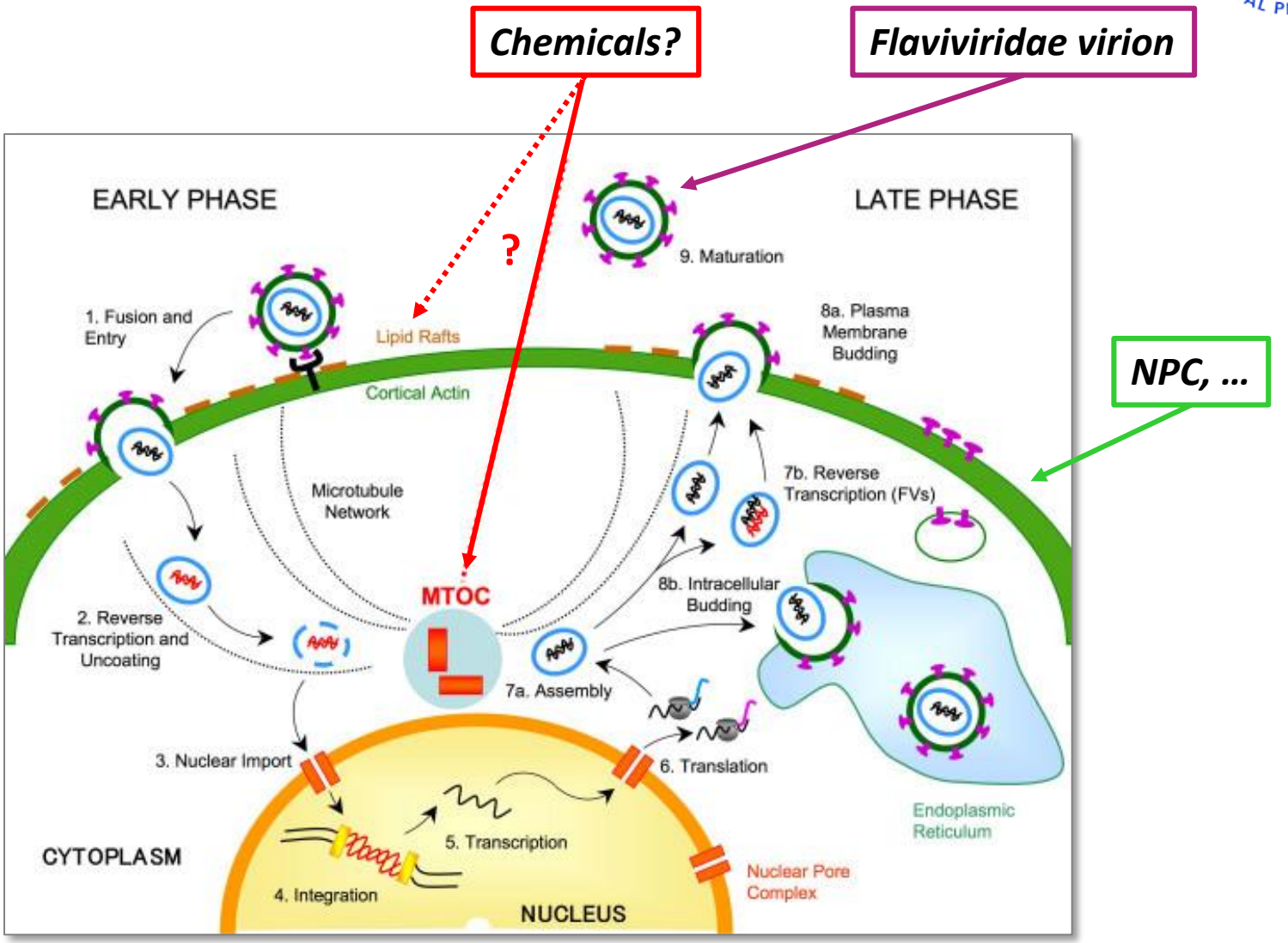


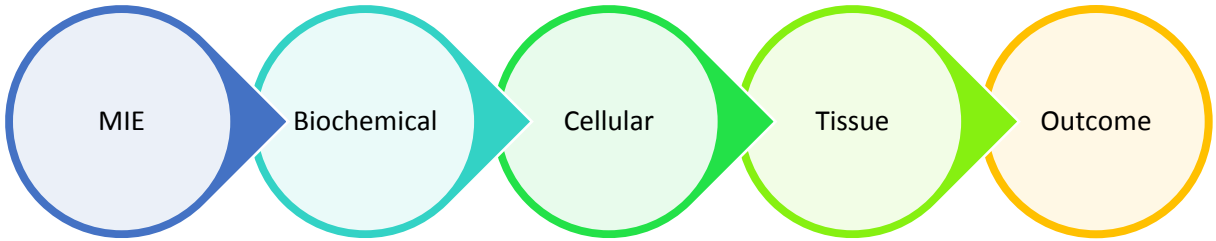


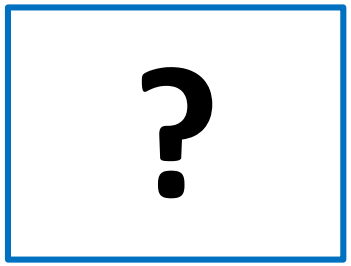
Biochemical Pathway: *centrosome cycle*

- One place to look is candidate molecules in genetic microcephaly, either as direct (primary) or secondary (e.g., paracrine, exosomes) effects of a stressor.
- Candidate genes for primary microcephaly in humans function in cellular pathways that regulate centrosome assembly and orientation [12].
- Key checkpoint is 'microcephalin' (MCPH1) working in concert with microtubule assembly and the cytoskeletal cycle (ASPM, CD5RAP2, CENJ, STIL).
- The centrosome cycle (centriole, microtubules, …) is also coupled to the DNA damage response in addition to its role in neurogenesis.

Microtubule-Organizing Center (MTOC)
(and the endocytic pathway)

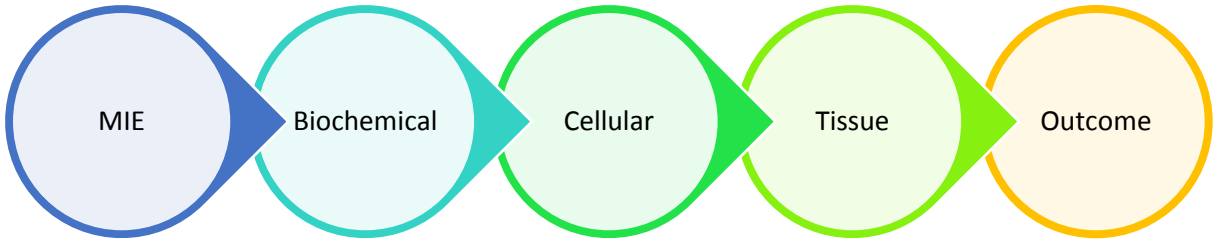


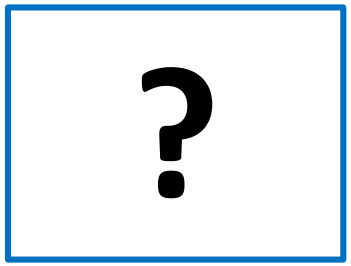




Molecular Initiating Event: *Unclear!*

- Specific receptors that bind drugs, chemicals, or virions are key to mechanistic understanding of cellular susceptibilities and therapeutic strategies.
- A place to look is biokinetics of target-cell susceptibility, such as expression and specificity of candidate receptors or enzyme targets.
- For example, AXL has been recently identified as the candidate viral entry factor and is enriched in radial glia, brain capillaries, microglia, and NPCs [13].
- The 'radial glia', which organize development of the blood brain barrier as well as the NPC architecture, may be the first target for ZIKV upon CSF uptake.

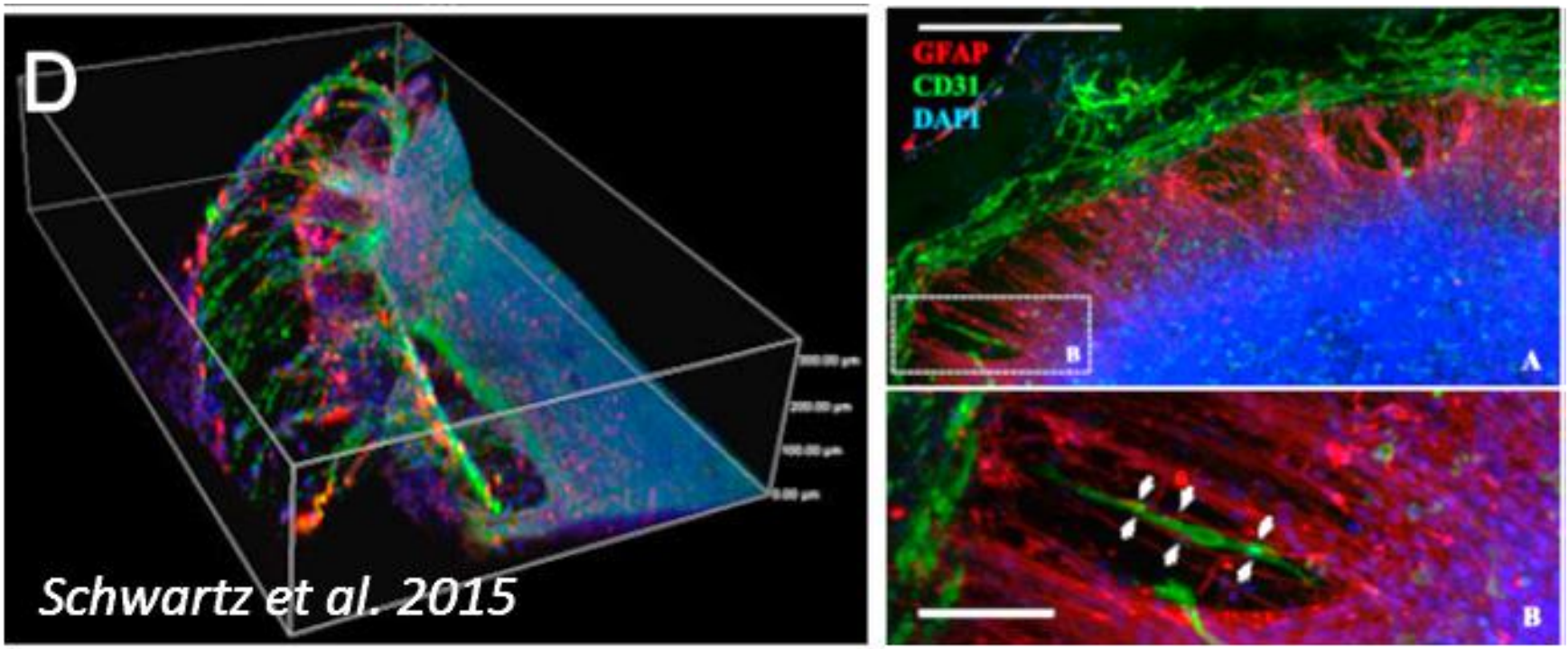




Molecular Initiating Event: *Unclear!*

- Opportunity for research and development of models that can be broadly applied to chemicals, viral infections and other factors in microcephaly.
- Cycle back to the 75 ToxRefDB chemicals that affect brain development and look for bioactivity profiles in ToxCastDB.
- predominant focus to date for the current Tox21 collaboration has been on developing and applying high-throughput screening to toxicology
- Limitations include lack of xenobiotic metabolism, inability to screen volatile or highly hydrophilic chemicals, limited coverage of biological targets, and estimation of effect levels based on nominal concentrations.

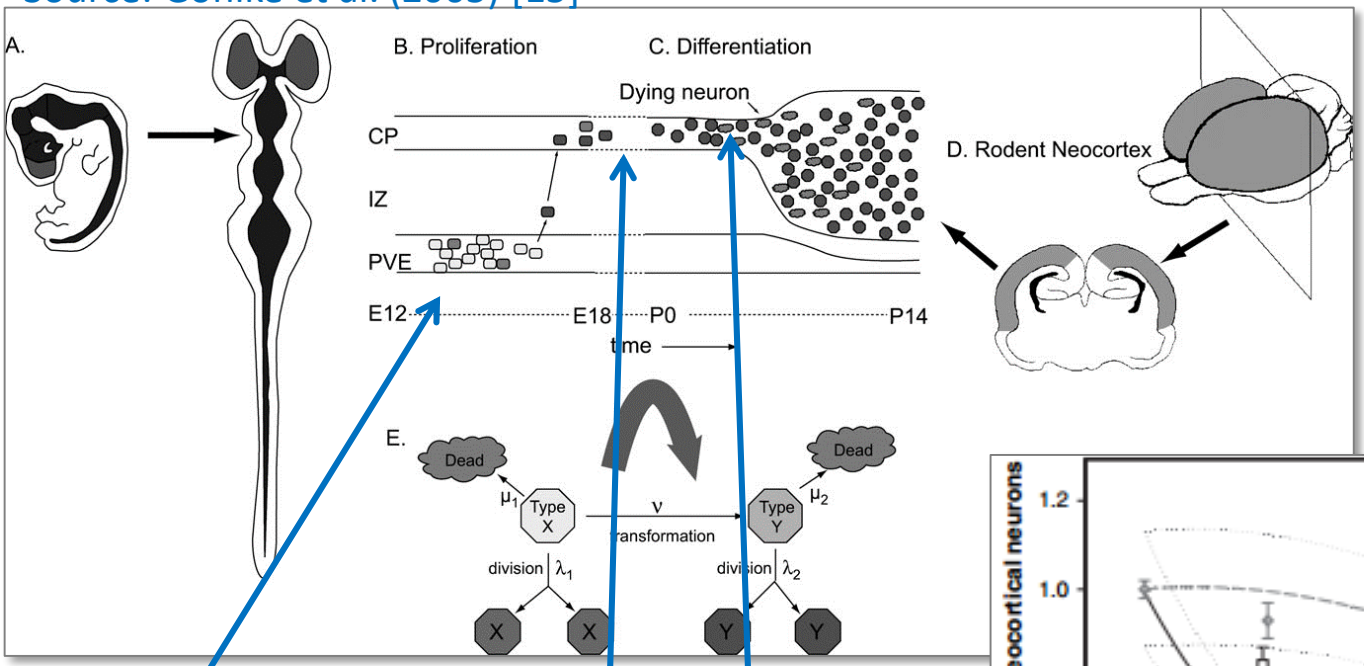
Modeling the system with human brain mimics



- Vascularized 3D organoids developed from hNPCs + iEndothelia + microglia [14].
  - models to evaluate DNT as part of STAR Center (EPA-G2013-STAR-L1/835737)
- Such models can be used to gain fundamental understanding of the cellular and genomic changes to build on the AOP for microcephaly in a human system.

Modeling the system mathematically

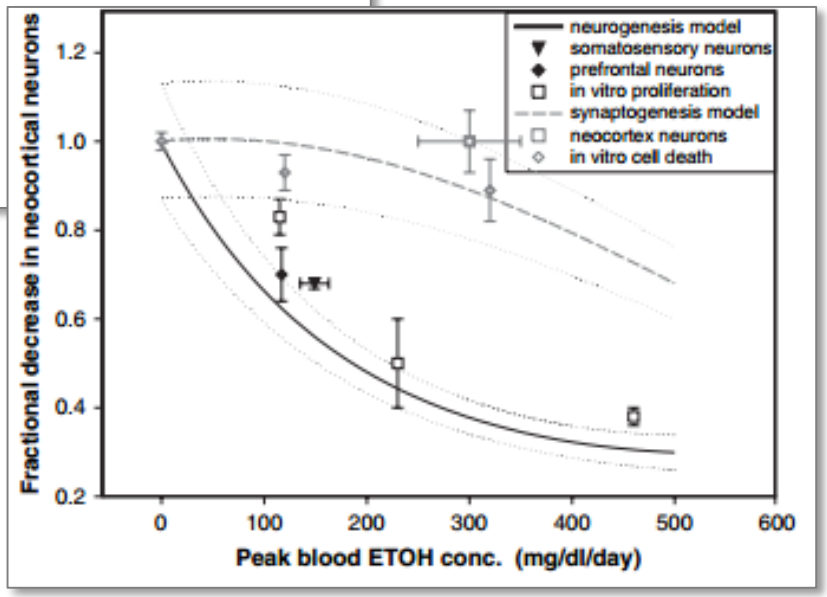
Source: Gohlke et al. (2005) [15]



NPCs in ventricular zone

Neurogenic switch

Apoptotic cell



Anatomical homeostasis in a self-regulating multicellular system

SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

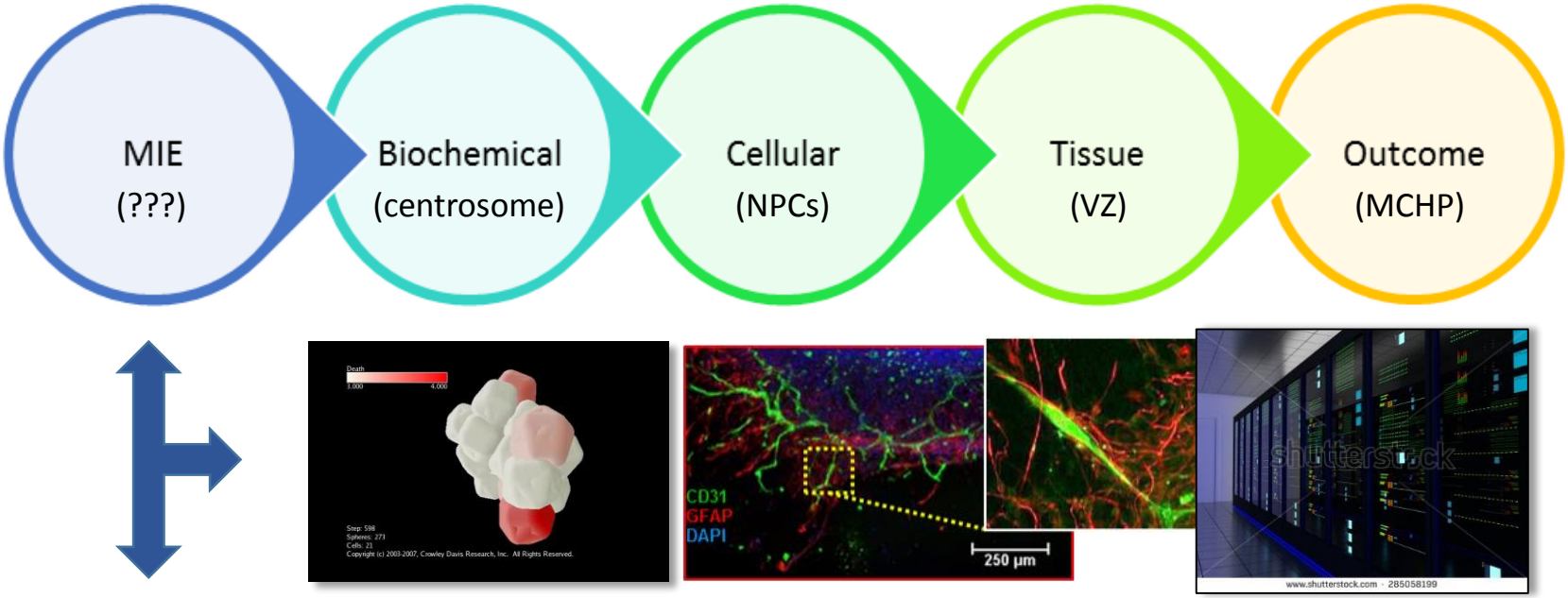
Can a computer model of the developing embryo translate cellular disruptions into a prediction of dysmorphogenesis?

and if so …

How might such models be used with high-performance computing **analytically** (to understand) and **theoretically** (to predict) adverse developmental outcomes for different exposure scenarios?

e.g., chemicals, non-chemical stressors, drugs, mixtures, lifestages, …

Summary



ToxCast bioactivity signature	cellular dynamics in computer simulation	tissue responses in human brain mimics	AOP-based hazard prediction model

GOAL: systems models for use with high-performance computing (to predict) and in vitro models (to validate) biological processes underlying pathogenesis of this complex defect.

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