JOHNS HOPKINS
BLOOMBERG SCHOOL
of PUBLIC HEALTH

THE CHALLENGE OF DISEASE MODELING, QUALITY ASSURANCE AND VALIDATION OF ORGAN-ON-CHIP MODELS

THOMAS HARTUNG, MD, PHD

DOERENKAMP-ZBINDEN CHAIR, EVIDENCE-BASED TOXICOLOGY

MOLECULAR BIOLOGY & IMMUNOLOGY

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Consulting VP of Scientific Affairs

AxoSim

Conflicts of Interest

AxoSim
Human Data, Faster.

Consulting VP
shareholder

AstraZeneca

Consultant

ATCC®

inSphero

MERCK MILLIPORE

Licensed Pyrogen Test
Consultant

UL

Consultant
Comp. Tox.

Green Chemistry
Advisory Panel

ToxTrack

Consultant, shareholder
In preparation: Insilica LLC

Let's not beat a dead horse* talking once again about the shortcomings of animal tests

- For the 9 most common OECD tox tests, reproducibility is 81%, 69% for toxic substances
- Mice and rat predict each other ~60%

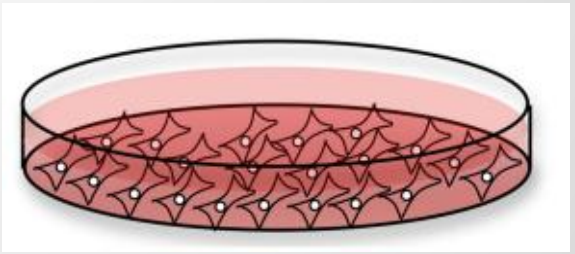
*Completely inappropriate for CAAT

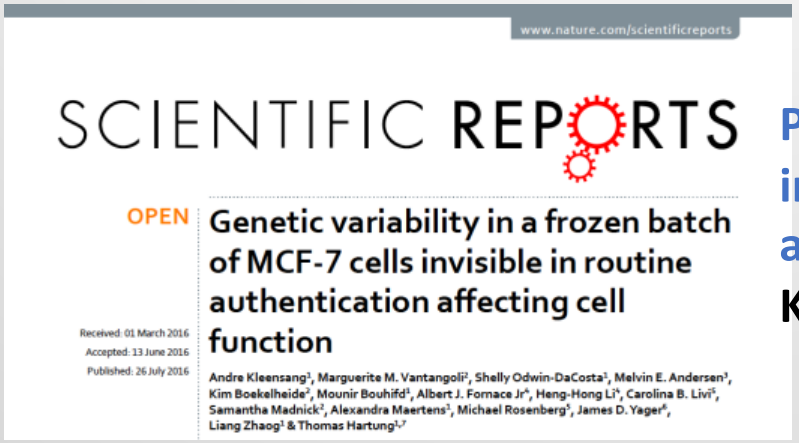
But we have to talk about:

Irreprodu-*cell*-bility

Cell tests have not less problems!

- Ca. 25% of cell lines misidentified
- 15-25% mycoplasma infected
- Genetic instability
- Culture artifacts





www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Genetic variability in a frozen batch of MCF-7 cells invisible in routine authentication affecting cell function

Received: 01 March 2016
Accepted: 13 June 2016
Published: 26 July 2016

Andre Kleensang[1], Marguerite M. Vantangoli[2], Shelly Odwin-DaCosta[1], Melvin E. Andersen[1], Kim Boekelheide[2], Mounir Bouhifd[1], Albert J. Fornace Jr[4], Heng-Hong Li[1], Carolina B. Livi[1], Samantha Madnick[2], Alexandra Maertens[1], Michael Rosenberg[1], James D. Yager[4], Liang Zhaog[1] & Thomas Hartung[1,7]

Pronounced genetic differences in frozen cells of the same lot from a cell bank

Kleensang (2016) Sci Rep 6, 28994

Genetic instability

MCF-7 cells - ~42,000 articles

Comparative Genome Hybridization:

10% genome lost
50% of genes less than 2 copies
30% of genes more than 2 copies (up to 30)

Overcome by Stem cells

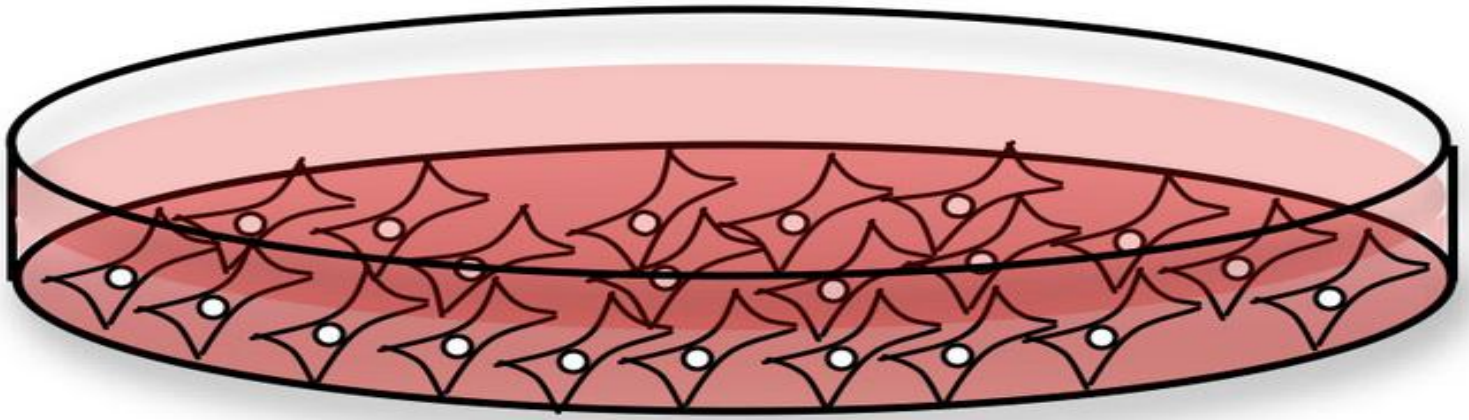
"Franken-cell"

Karyotyping

Traditional culture: pan-fried eggs "sunny side up"

Cell density ca. 0.1% of tissue,
Dilution of all secreted factors

Single cell types
No tissue architecture
No organ functionality



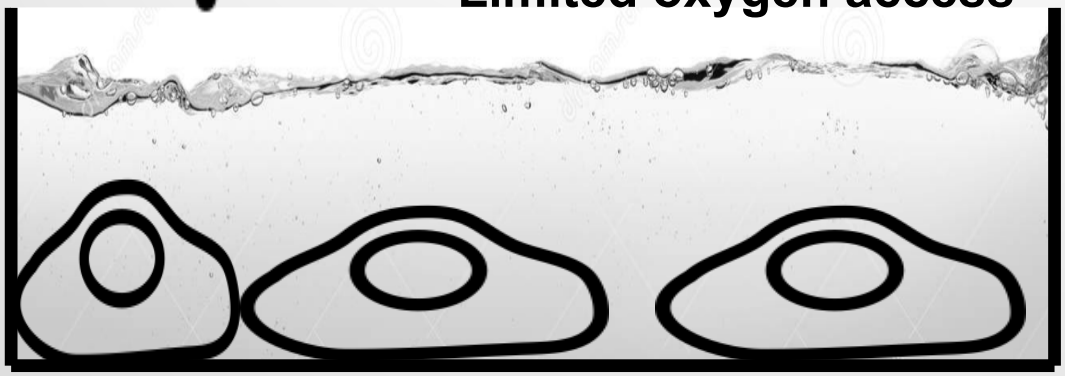
Cell to cell contact about 2%,
49% plastic, 49% medium

Overcome by 3D organoids

Sudden environment changes

Overcome by Perfusion (chip)

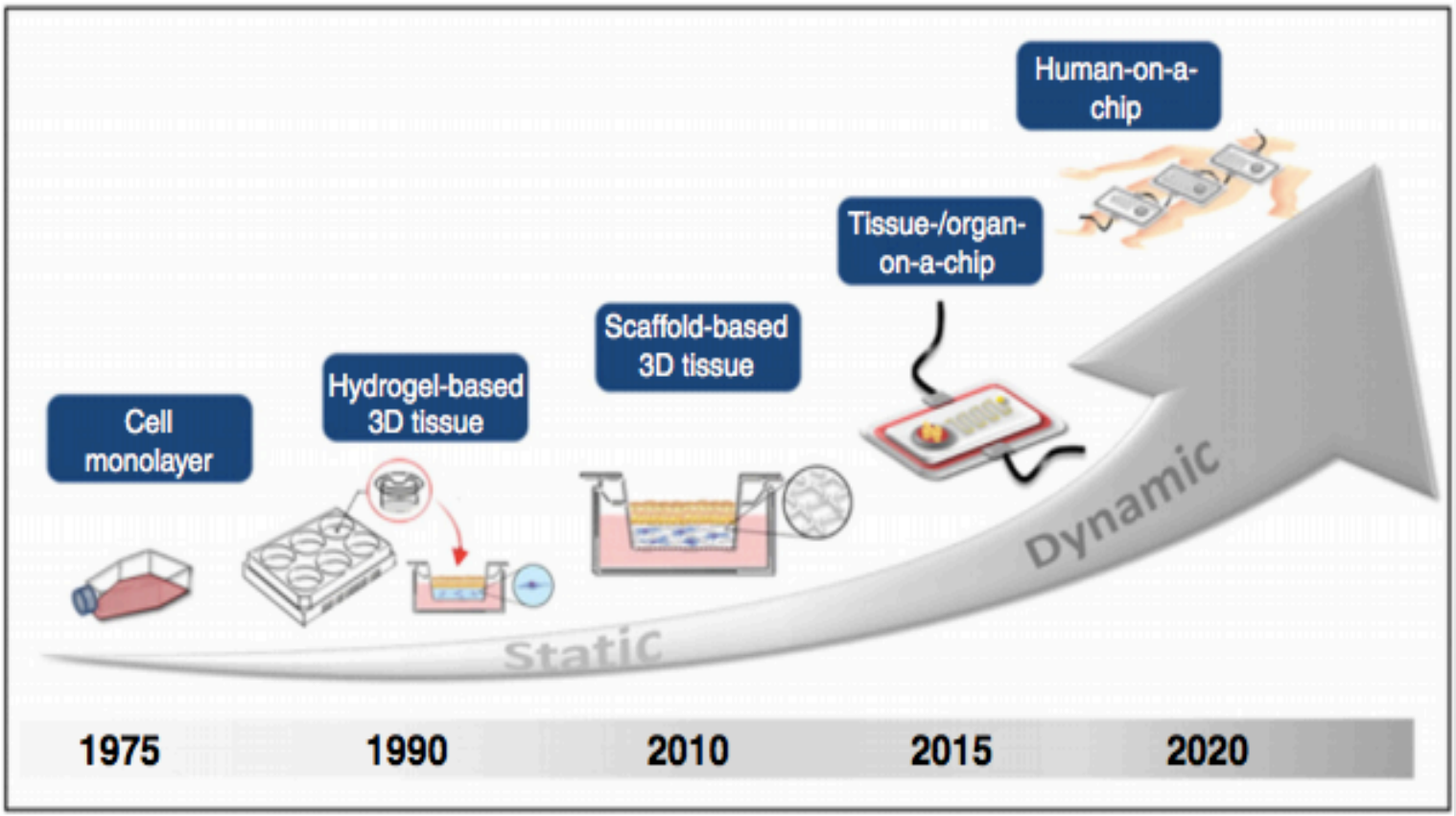
Non-physiological environment
Growth factors and excess nutrients
Limited oxygen access



Edge-effects
Minimal cell contacts
No demand on cell functions
Cell cross-contamination

No polarization,
No flow

Evolution of Cell Culture - high-tech & business opportunity



Marx et al. 2016

Current Opinion in Biotechnology

Marx et al., Biology-inspired micro-physiological system approaches to solve the prediction dilemma of substance testing using animals. ALTEX 2016, 33:272-321.

Marx et al., Biology-inspired microphysiological systems to advance medicines for patient benefit and animal welfare. ALTEX 2020, 37: in press.

Chemical Research in Toxicology

Chem Res Toxicol 2017, 30:43–52

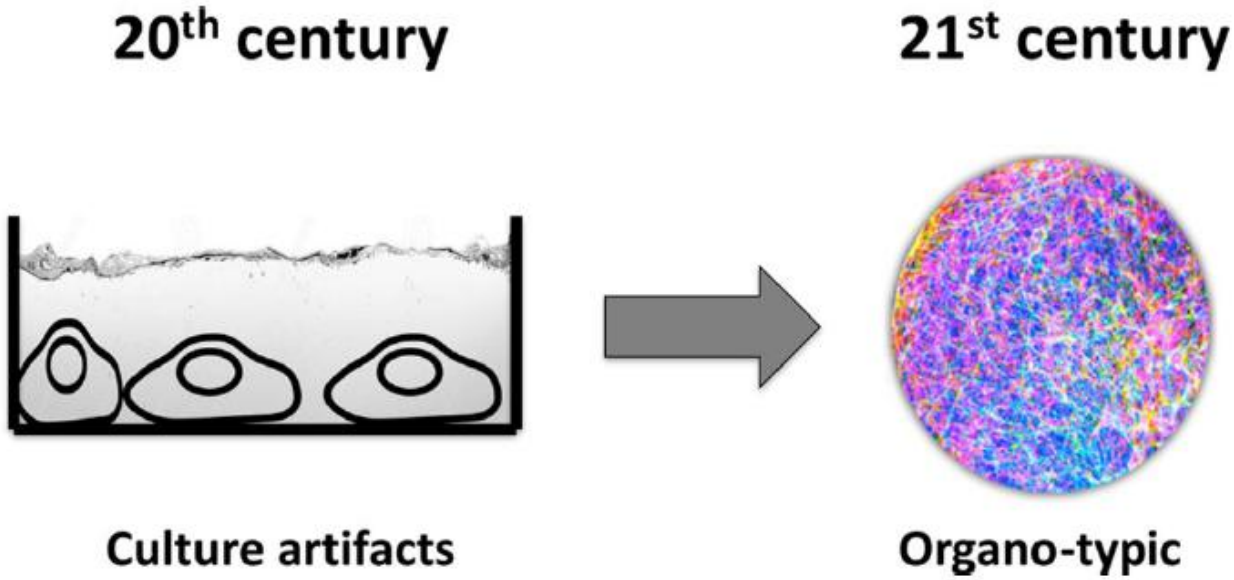
Perspective

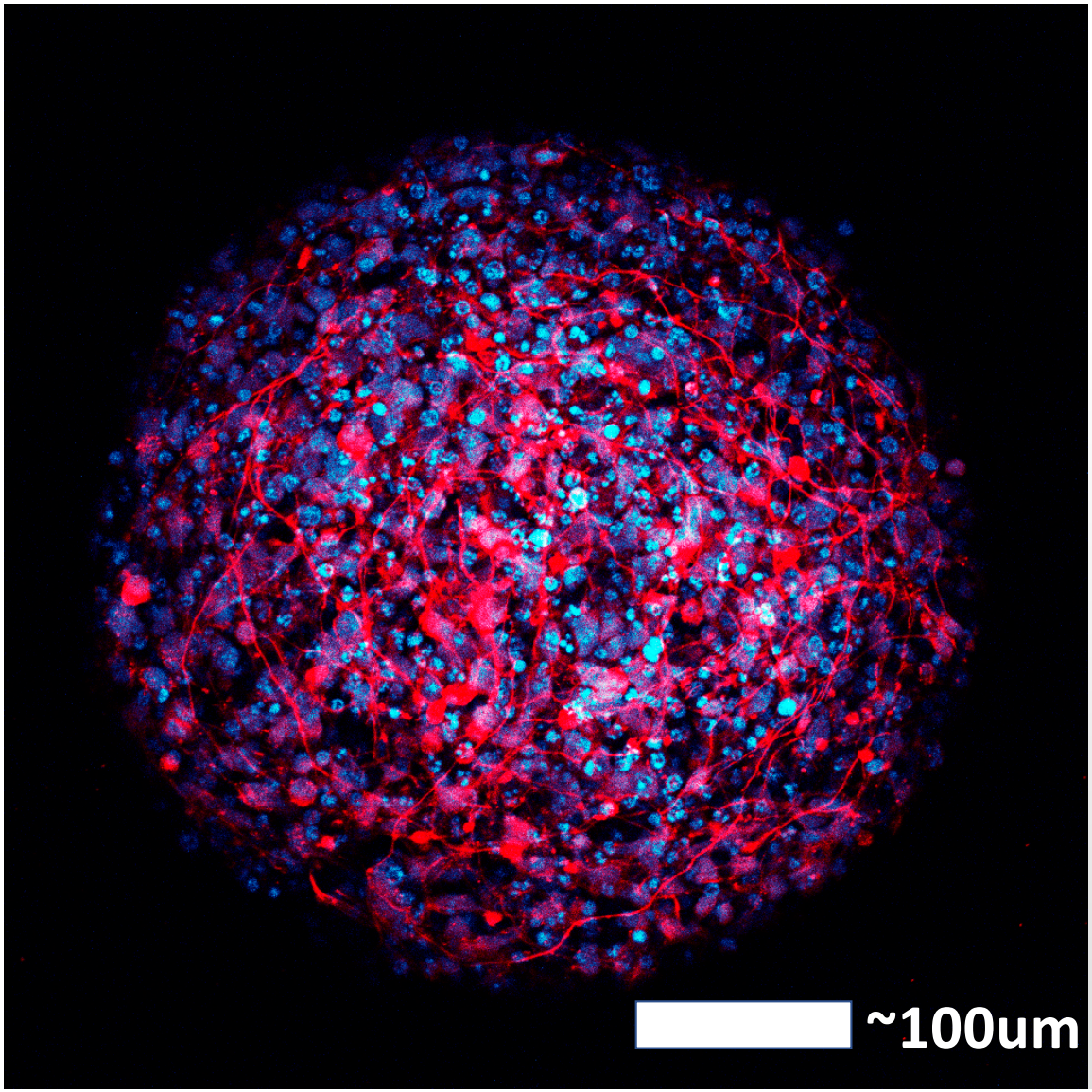
pubs.acs.org/crt

Life Sciences

21st Century Cell Culture for 21st Century Toxicology

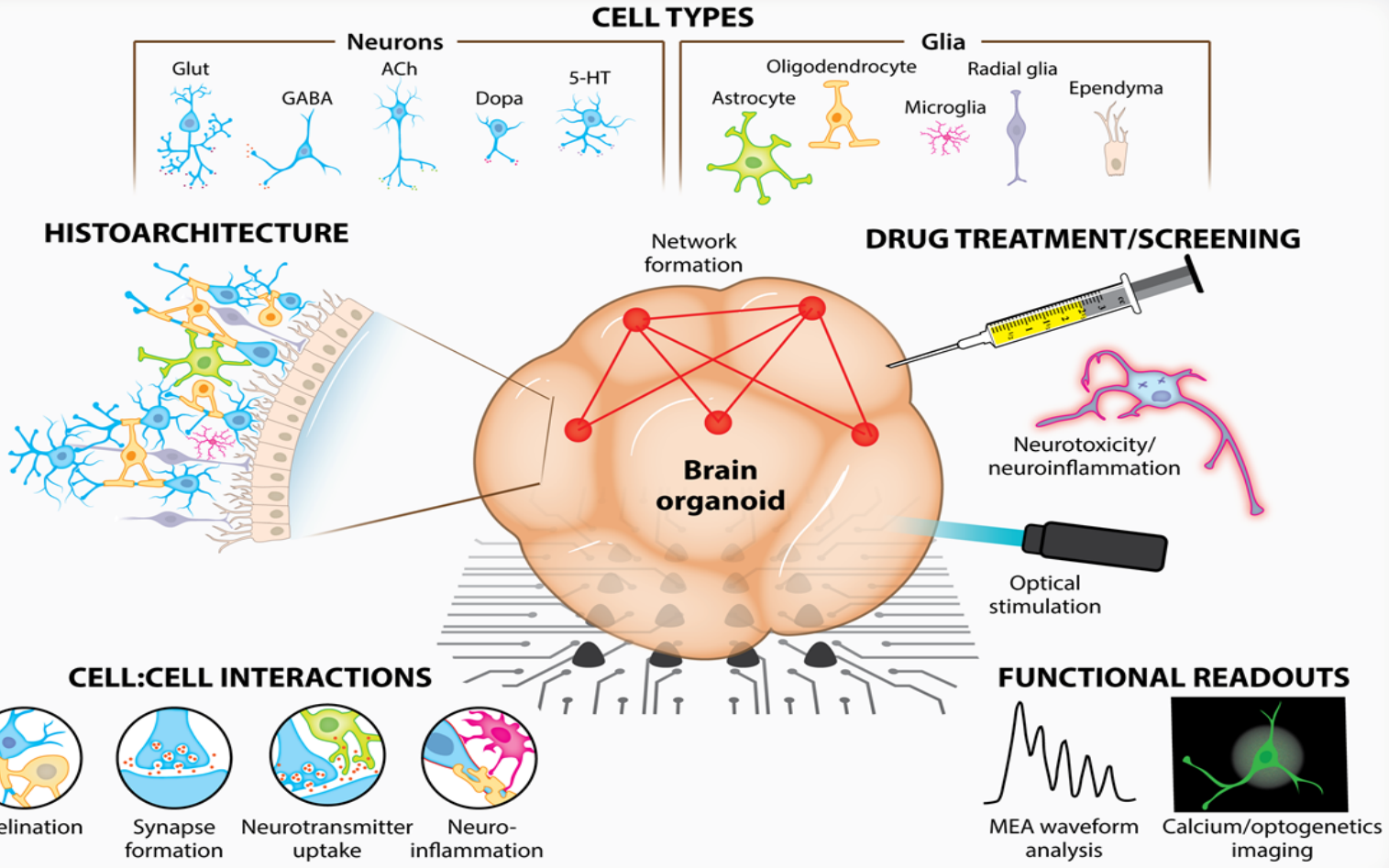
David Pamies[†] and Thomas Hartung*[,†,‡]





CAAT's BrainSphere Project

- From skin of donors, individual stem cells
- In 3 months thousands of identical organoids
- Neurons communicating
- Some brain functionality

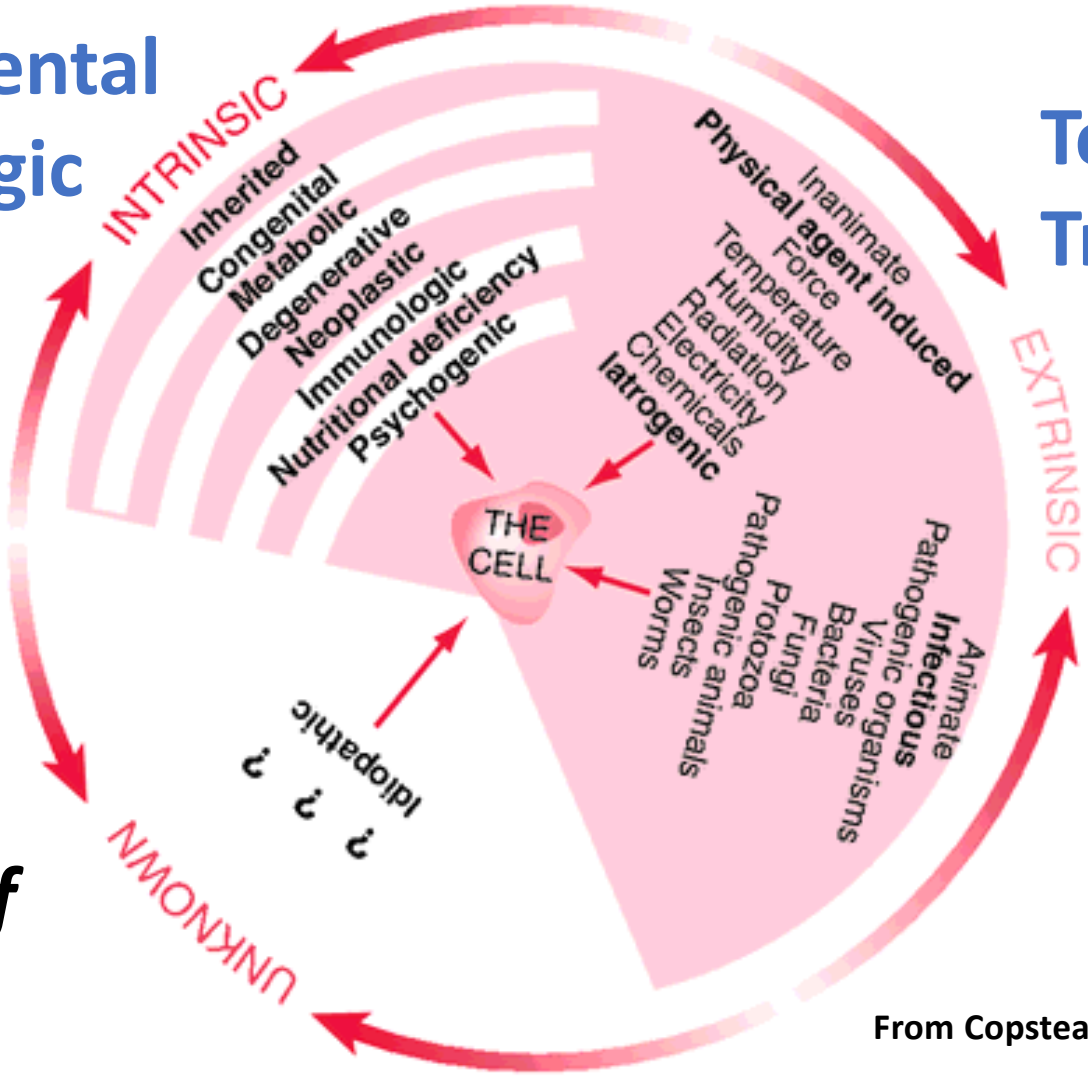


Anderson et al., In Vitro Cellular & Developmental Biology 2021, Published online. Doi: 10.1007/s11626-020-00532-8

The next challenge is to model disease:
From microphysiological to
***micropathophysiological** models*

Petri-dishes with different disease models

Developmental
Immunologic
Cancer
Genetics



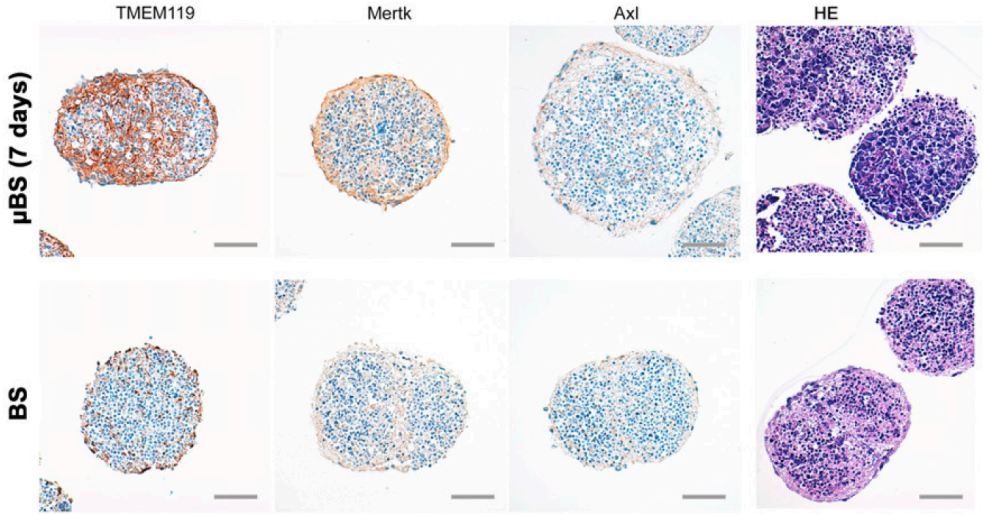
Toxicity
Trauma

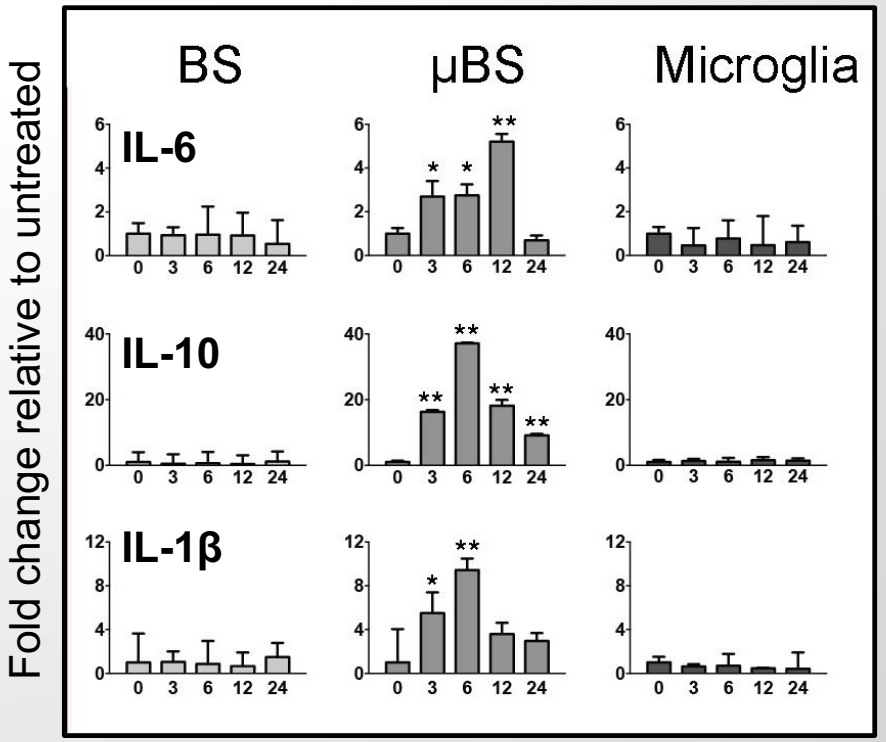
Infection

Etiology of diseases

From Copstead and Banasik, 2000.

Microglia in BrainSpheres to study neuroinflammation





Hours post-treatment with LPS

Similar: Dengue & ZIKA virus

frontiers in Microbiology

ORIGINAL RESEARCH
published: 04 December 2018
doi: 10.3389/fmicb.2018.02766

Microglia Increase Inflammatory Responses in iPSC-Derived Human BrainSpheres

*Celina Monteiro Abreu[1], Lucio Gama[1,2], Susanne Krasemann[3], Megan Chesnut[4], Shelly Odwin-Dacosta[4], Helena T. Hogberg[4], Thomas Hartung[4,5] and David Pamies[4]**

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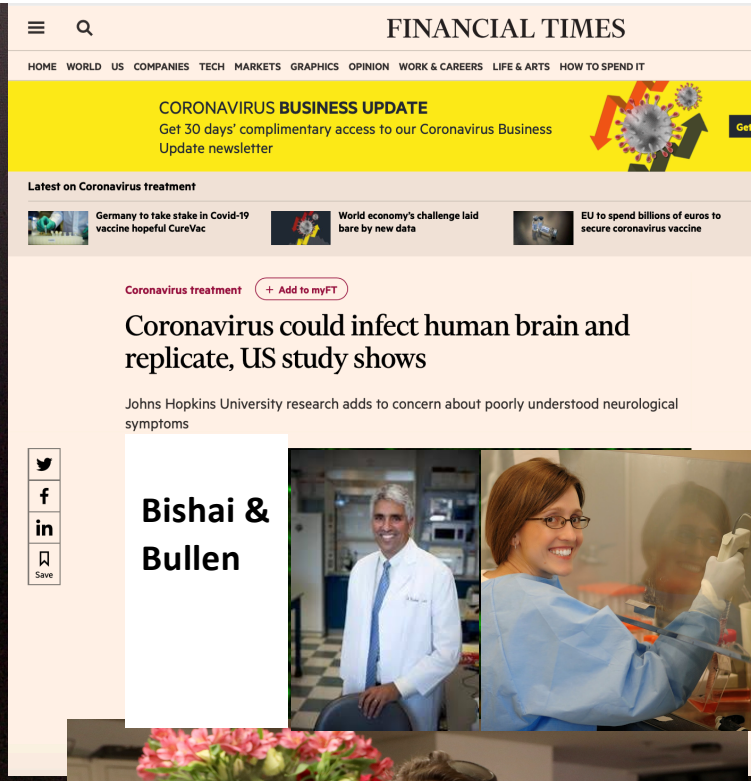
A SARS-2-CoV-infected human brain cell



Bishai & Bullen

Hogberg, Sillé & Smirnova

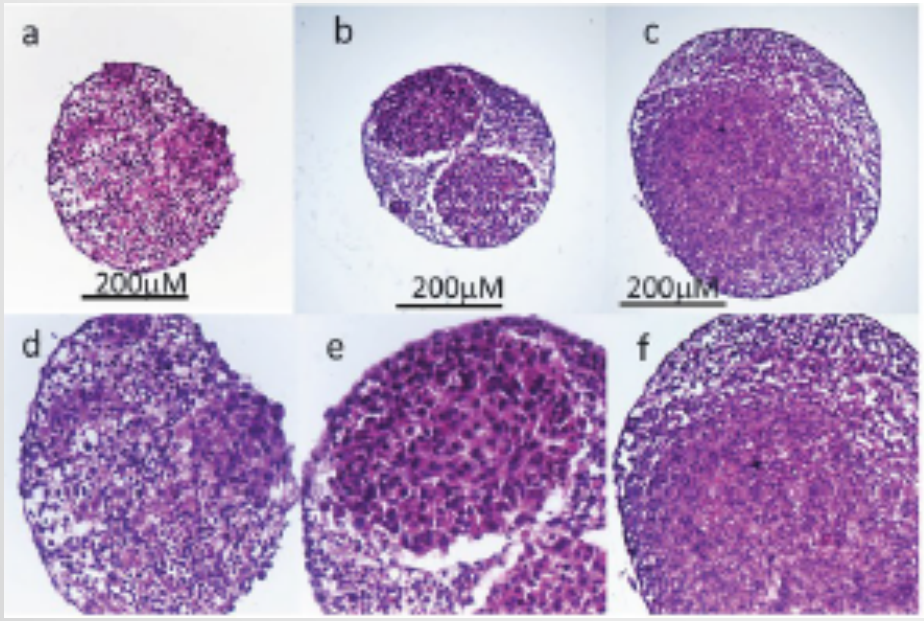
Short Communication

ALTEX 2020, 37:665-671

Infectability of Human BrainSphere Neurons Suggests Neurotropism of SARS-CoV-2

C. Korin Bullen[#1], Helena T. Hogberg[#2], Asli Bahadirli-Talbott[1], William R. Bishai[1], Thomas Hartung[2,3,4], Casey Keuthan[5], Monika M. Looney[1], Andrew Pekosz[4], J. Carolina Romero[2], Fenna C. M. Sillé[2,6], Peter Um[1] and Lena Smirnova[2,#]

Glioblastoma in BrainSpheres



Brain with tumor

Effect of Temozolomide and Doxorubicin treatment

SCIENTIFIC REPORTS

OPEN

A Human iPSC-derived 3D platform using primary brain cancer cells to study drug development and personalized medicine

: 22 August 2018
: 19 December 2018
d online: 05 February 2019

Simon Plummer[1], Stephanie Wallace[1], Graeme Ball[2], Roslyn Lloyd[3], Paula Schiapparelli[4], Alfredo Quiñones-Hinojosa[4], Thomas Hartung[5,6] & David Pamies[5,7]

- **DEVELOP DRUGS**
- **OPTIMIZE CHOICE OF DRUG**

Toxicology and Applied Pharmacology 354 (2018) 101–114

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Rotenone exerts developmental neurotoxicity in a human brain spheroid model

David Pamies[a], Katharina Block[a], Pierre Lau[b], Laura Gribaldo[b], Carlos A. Pardo[c], Paula Barreras[c], Lena Smirnova[a], Daphne Wiersma[a], Liang Zhao[a,d], Georgina Harris[a], Thomas Hartung[a,e], Helena T. Hogberg[a,*]

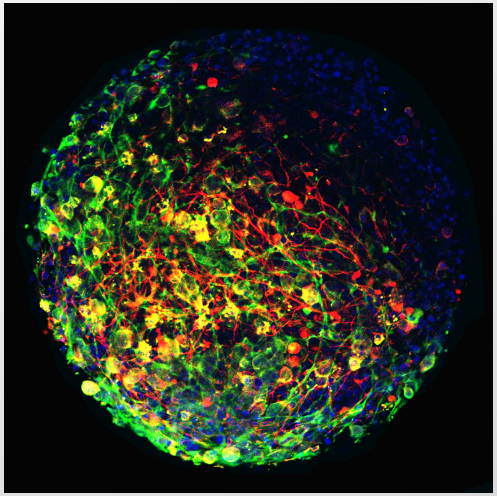
In conclusion, our BrainSpheres model has shown to be a reproducible and novel tool to study neurotoxicity and developmental neurotoxicity. Results presented here support the idea that rotenone can potentially be a developmental neurotoxicant.

frontiers

Antidepressant Paroxetine exerts developmental neurotoxicity in an iPSC-derived 3D human brain model

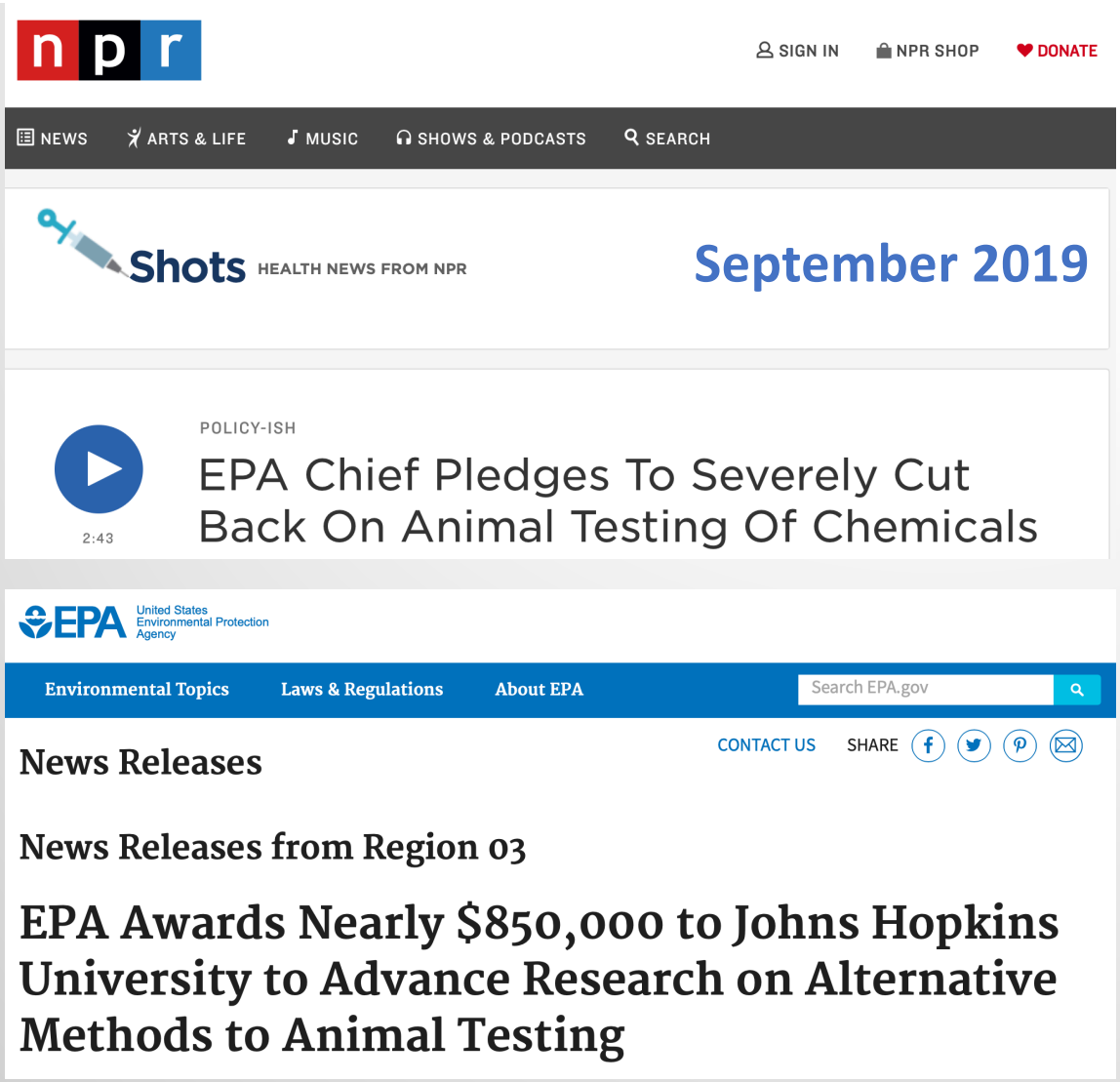
Xiali Zhong[1, 2], Georgina Harris[1], Lena Smirnova[1], Valentin Zufferey[3], Rita Sa[4], Fabiele Baldino Russo[5], Patricia C. Baleeiro Beltrao Braga[5], Megan Chesnut[1], Marie-Gabrielle Zurich[3], Helena Hogberg[1], Thomas Hartung[6, 7], David Pamies[3, 1*]

The model identifies suspected developmental neurotoxicants and models various diseases



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

EPA-funded development of DNT assay



Multiplexed human BrainSphere Developmental Neurotoxicity test for six key events of neural development

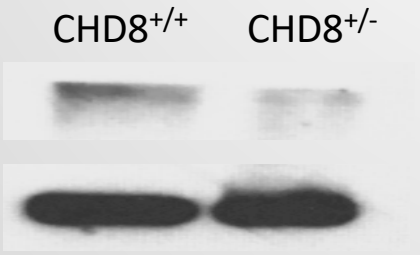
Smirnova, Hartung, Berlinicke, Gracias

CHD8 knockout BrainSpheres (CRISPR-CAS9) as disease model

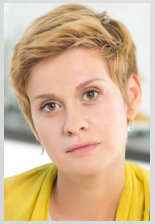
iPSC carrying CHD8 mutation along with control cell line

BrainSpheres from iPSC with k/o CHD8

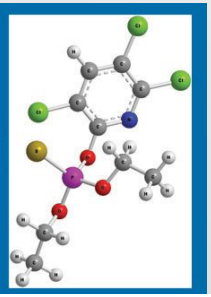
$CHD8^{+/+}$ - control iPSC
$CHD8^{+/-}$ - heterozygous knockout



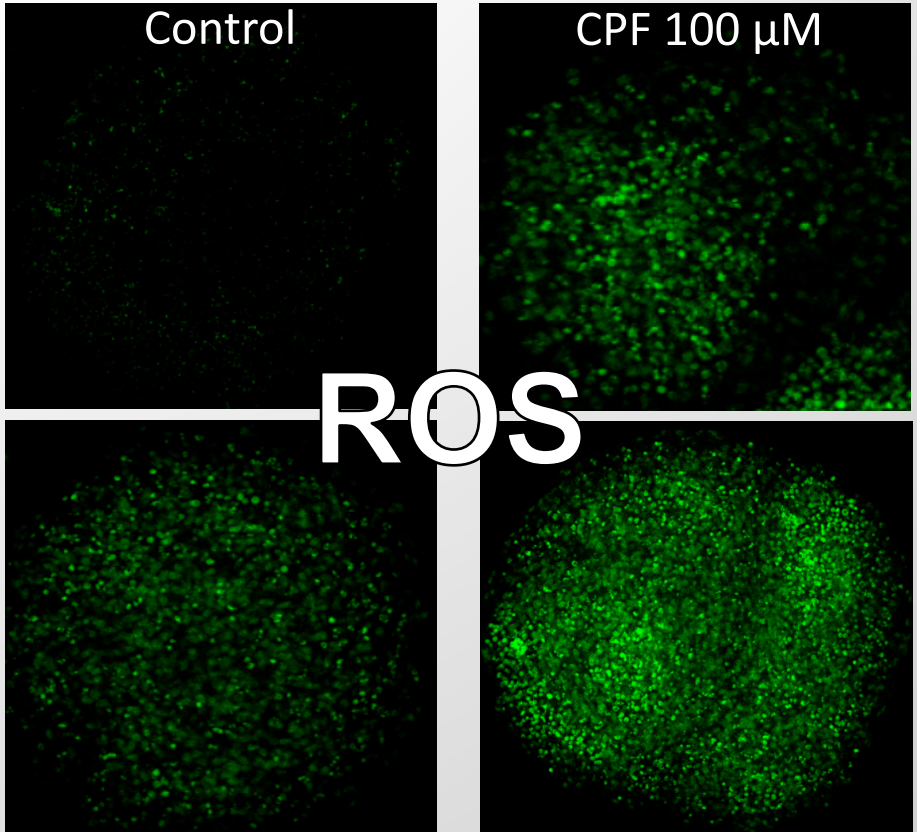

Lachman


Smirnova

CHLORPYRIFOS
CHLORPYRIFOS-OXON



unpublished



MPS WORLD SUMMIT
CONNECT, EXCHANGE, EDUCATE

~35 organizations
Scientific Advisory Board

New date!

Virtual Jun & Dec 2021
New Orleans, Jun 2022
Hosts: Suzie Fitzpatrick, FDA
Thomas Hartung, Hopkins
Don Ingber, Harvard

Letter

ALTEX 2020, 37: 490-492

Good Cell and Tissue Culture Practice 2.0 (GCCP 2.0) – Draft for Stakeholder Discussion and Call for Action

David Pamies[1], Marcel Leist[2,3], Sandra Coecke[4], Gerard Bowe[4], Dave Allen[5], Gerhard Gstraunthaler[6], Anna Bal-Price[4], Francesca Pistollato[4], Rob deVries[7,8], Thomas Hartung[2,9] and Glyn Stacey[10,11,12]

GCCP 2.0

Draft published

- **Stakeholder discussion**
- **Editor workshop**
- **Funding bodies**

Register at:

CAAT@jhu.edu

ALTEX 2019, 36:3-17

"In God we trust.
All others must bring data."
W. Edwards Deming
(1900-1993)
Professor and author

Food for Thought ...

Toward Good *In Vitro* Reporting Standards

Thomas Hartung [1,2], Rob de Vries [3], Sebastian Hoffmann [4], Helena T. Hogberg [1], Lena Smirnova [1], Katya Tsaioun [1], Paul Whaley [5] and Marcel Leist [2]

Bench Marks!

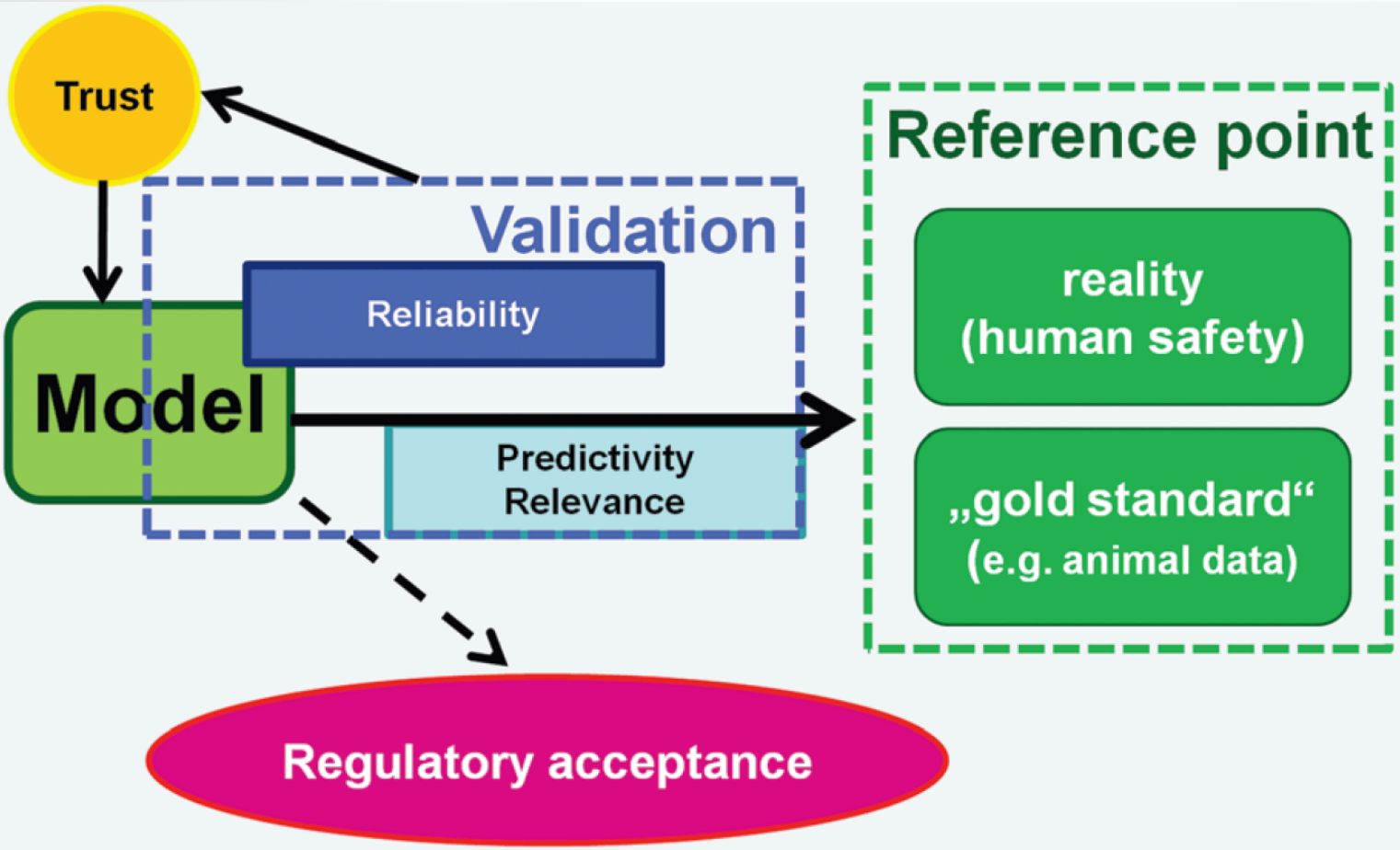
Template for the Description of Cell-Based Toxicological Test Methods to Allow Evaluation and Regulatory Use of the Data

Alice Krebs[1,2], Tanja Waldmann[1], Martin F. Wilks[3], Barbara M. A. van Vugt-Lussenburg[4], Bart van der Burg[4], Andrea Terron[5], Thomas Steger-Hartmann[6], Joelle Ruegg[7], Costanza Rovida[8], Emma Pedersen[9], Giorgia Pallocca[1,8], Mirjam Luijten[10], Sofia B. Leite[11], Stefan Kustermann[12], Hennicke Kamp[14], Julia Hoeng[14], Philip Hewitt[15], Matthias Herzler[16], Jan G. Hengstler[17], Tuula Heinonen[18], Thomas Hartung[8,19], Barry Hardy[20], Florian Gantner[21], Ellen Fritsche[22], Kristina Fant[9], Janine Ezendam[10], Thomas Exner[20], Torsten Dunkern[23], Daniel R. Dietrich[24], Sandra Coecke[11], Francois Busquet[8,25], Albert Braeuning[26], Olesja Bondarenko[27], Susanne H. Bennekou[28], Mario Beilmann[29] and Marcel Leist[1,2,8]

The next GCCP project

ALTEX 2019, 36:682-699

What formal validation is about



Provide regulators the evidence whether they can trust a new / alternative method

Required only for regulatory methods, but helpful for any test

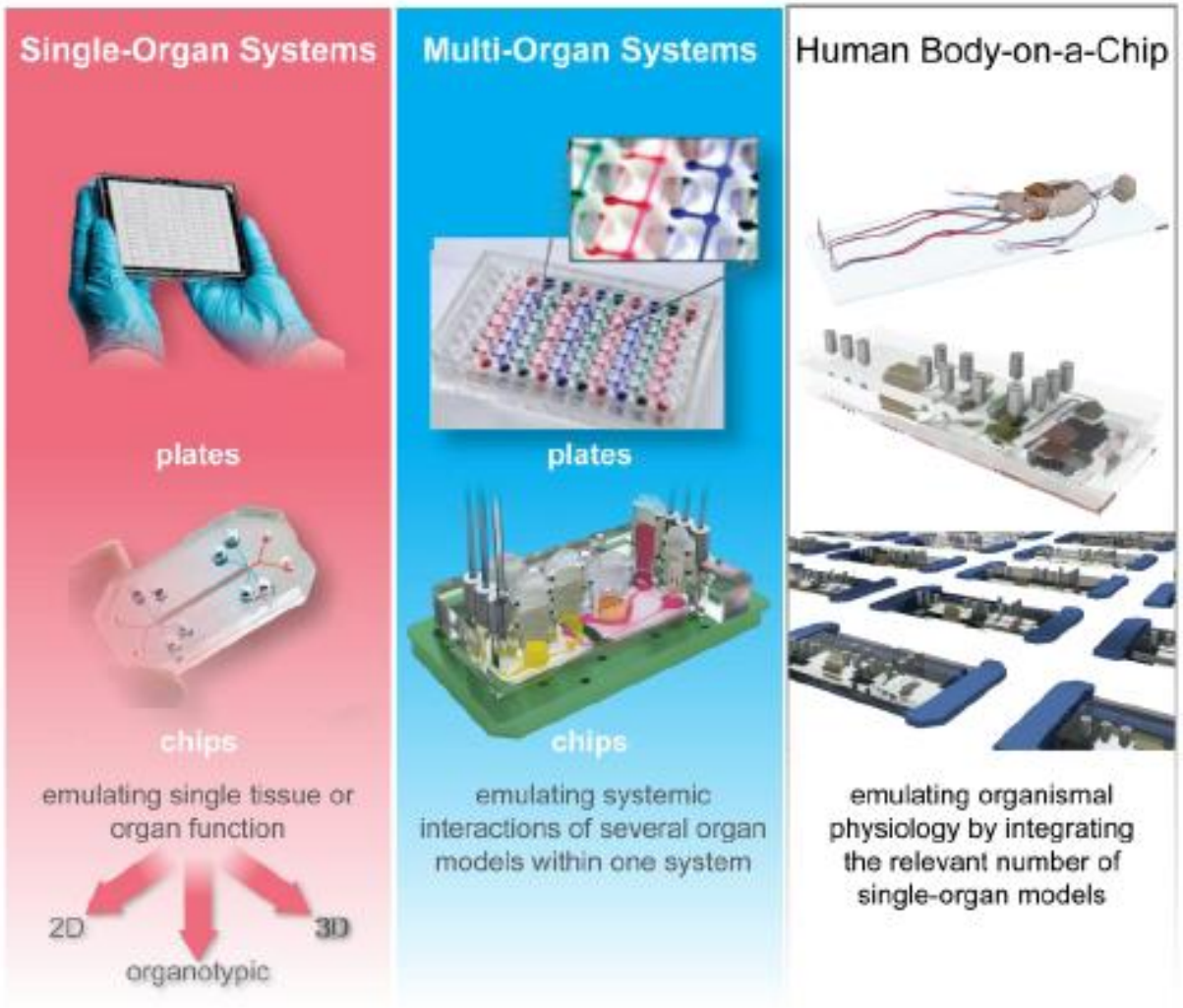


Fig. 3: Types of MPS used for emulation of human biology *in vitro*

Good luck, validating this in ring trials!

- **Number of variables**
- **Moving targets**
- **Costs and low throughput**
- **Lack of reference points**
- **Freezing in time in an area of fast change**

We need to adapt Validation

ALTEX 27 (2010) 253-263

Evidence-Based Toxicology – the Toolbox of Validation for the 21st Century?

Thomas Hartung
Johns Hopkins University, Bloomberg School of Public Health, Dept. Environmental Health Sciences, Center for Alternatives to Animal Testing (CAAT), Doerenkamp-Zbinden Chair for Evidence-based Toxicology, Baltimore, MD, USA, and Professor of Pharmacology and Toxicology, University of Konstanz, Germany

ALTEX 30 (2013) 119-130

Food for Thought ... Mechanistic Validation

Thomas Hartung [1,2], *Sebastian Hoffmann* [2,3], *and Martin Stephens* [1]
[1]Johns Hopkins Bloomberg School of Public Health, Center for Alternatives to Animal Testing (CAAT), Baltimore, MD, USA; [2]University of Konstanz, CAAT-Europe, Germany; [3]seh consulting, Paderborn, Germany

The difficulty lies not in the new ideas,

but in escaping from the old ones.

John Maynard Keynes

(1883 - 1946)