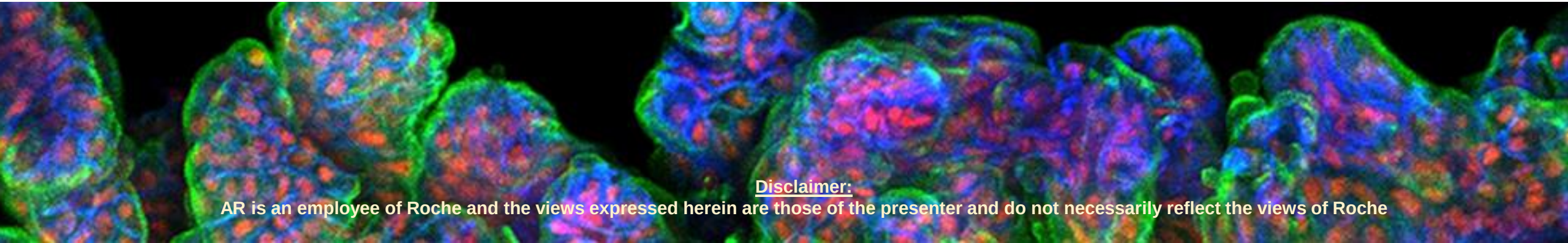
Danish 3R-symposium
Nov 17 2021, 12.30-13.10

Novel human cell models in drug development:

How 3D, Organoids & Organs on Chips can improve and renew current paths - and our vision for the future

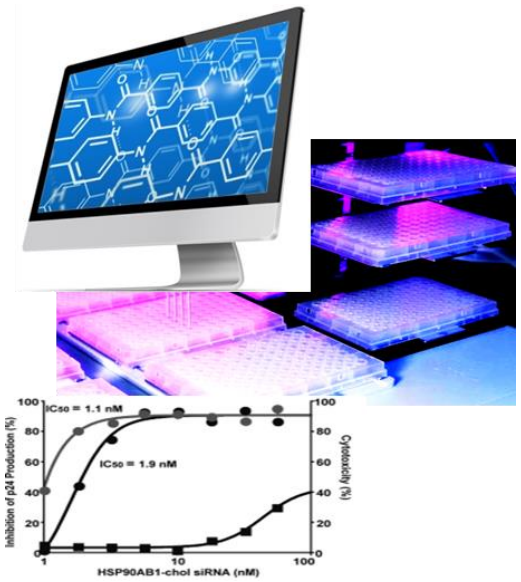
Prof. Adrian Roth, PhD
Principal Scientific Director
Personalized Healthcare Safety, Pharma Clinical Development
Hoffmann-La Roche Ltd, Basel, Switzerland



Disclaimer:
AR is an employee of Roche and the views expressed herein are those of the presenter and do not necessarily reflect the views of Roche

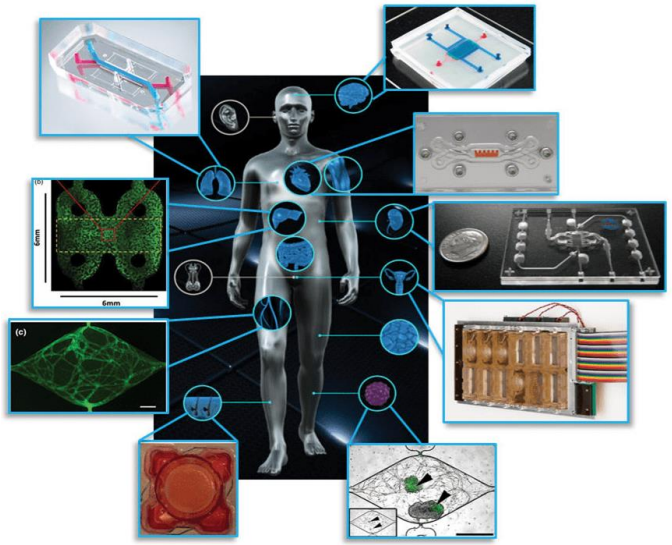
Efforts in Pharmaceutical Industry aiming at increasing Quality & Speed of Drug Development – while reducing Cost & Animal use

Fail early – fail fast



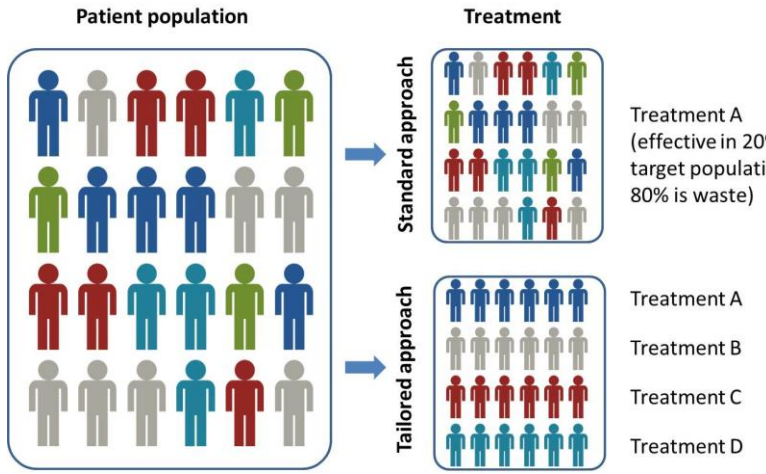
In silico design, lab automation, AI-powered algorithms

Organoids & "Organs on Chips"



Advanced human cell models that better recapitulate human physiology

"Real World Data" & Genetics



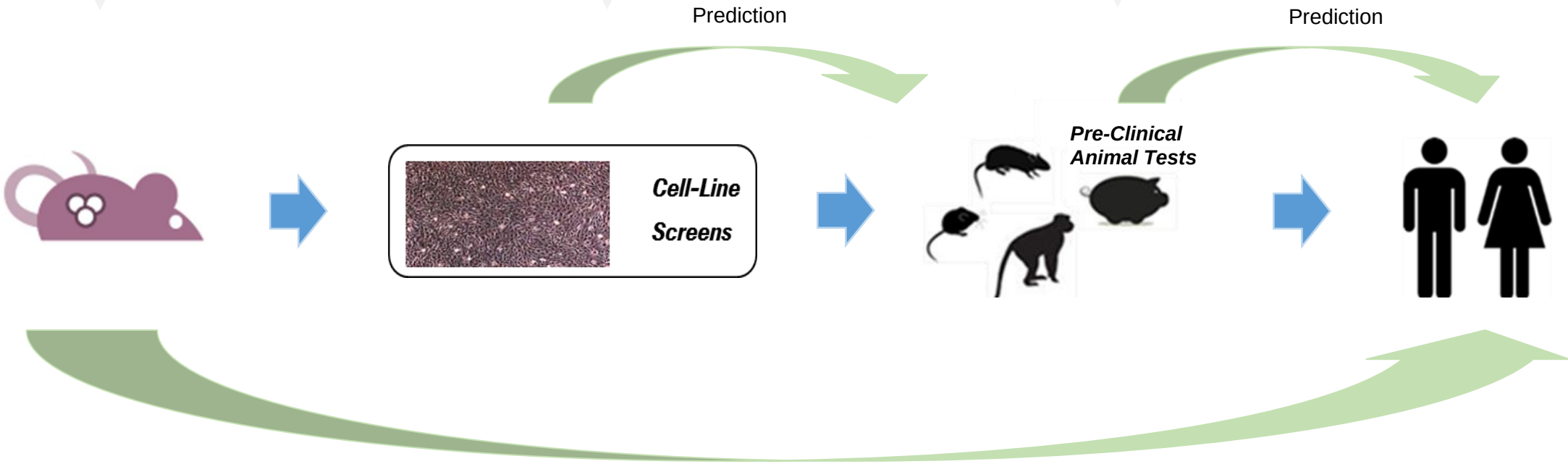
Personalized Medicine: Understand & predict human variability – optimize benefit/risk

Advanced Human Cell Models today

Support Disease modeling & Target Identification

Improve Candidate Selection before *in vivo*

Mechanistic Issue Resolution of Animal Findings

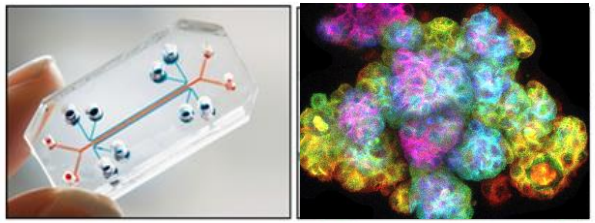


The Vision: Reverse-translate from human to *in vitro* to predict from *in vitro* to human

R&D in Bio-engineering & Cell Biology

Data Science Tools: AI, Predictive Algorithms

***In Vitro* Data & Human-relevant Cell Models**



Prediction

Anonymized Access to Patient Data

Reverse Translation

Real World Data & Human Tissue

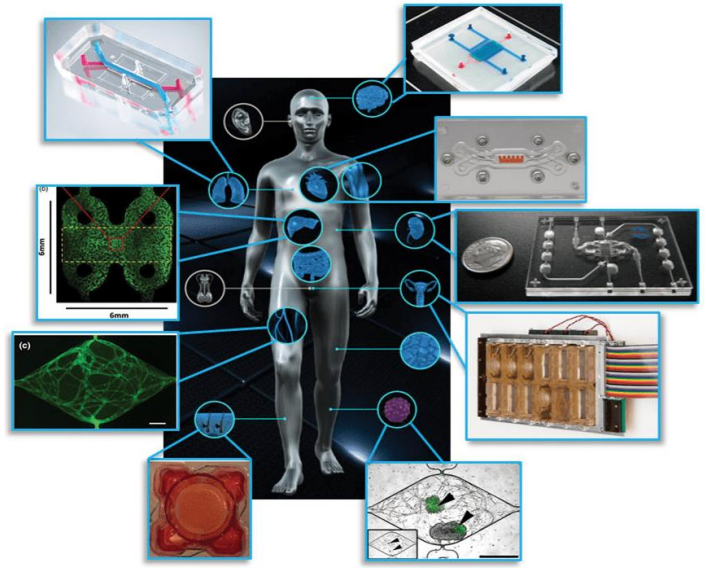
Data Science Tools: AI, Predictive Algorithms

Access to Patient-Tissue /-Stem Cells

Cooperation & Collaboration between Academia, Clinical Organizations, Patients/Patient Organizations, Data Science Experts, Biotech/Technolgy Start-Ups, Pharma, Regulators

Advanced human cell models in Pharmaceutical Drug Development: Where are we today ?

- **A burst of different approaches – some with more, some with less potential for application**
- **Not fully clear yet which cells, which materials, which set-ups, which assays or which endpoints are «the right ones»**
- **A lot of overviews & strategies published – yet, best and most convincing way for adoption is to demonstrate compelling evidence for defined use cases**
- **Broad industry adoption is slow – reasons may include**
  - **high upfront investments**
  - **complex technical setting**
  - **lack of robustness**
  - **lack of clear superiority over existing models**
  - **unclear translational potential to the human situation**



Opportunities and challenges with microphysiological systems: a pharma end-user perspective

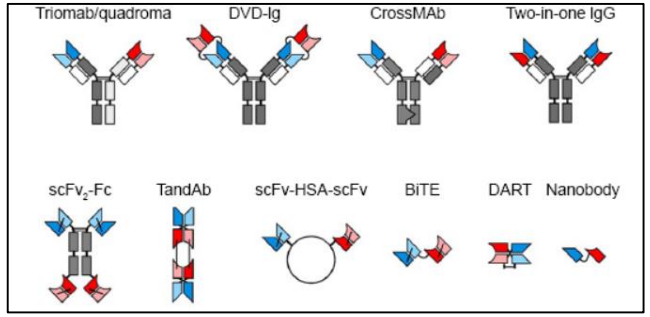
Lorna Ewart[1] and Adrian Roth[2]

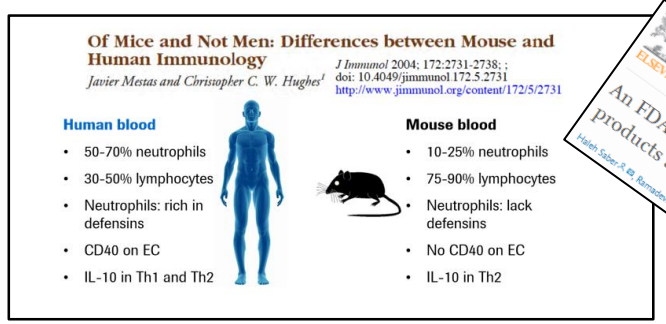
Using human-relevant, translational in vitro models is widely considered to reduce attrition during drug discovery and development. Despite this, the adoption of models based on microphysiological systems — organs-on-chips or organoids — by pharma companies is moderate at best, and realizing the full potential of these models will need greater collaboration between stakeholders.

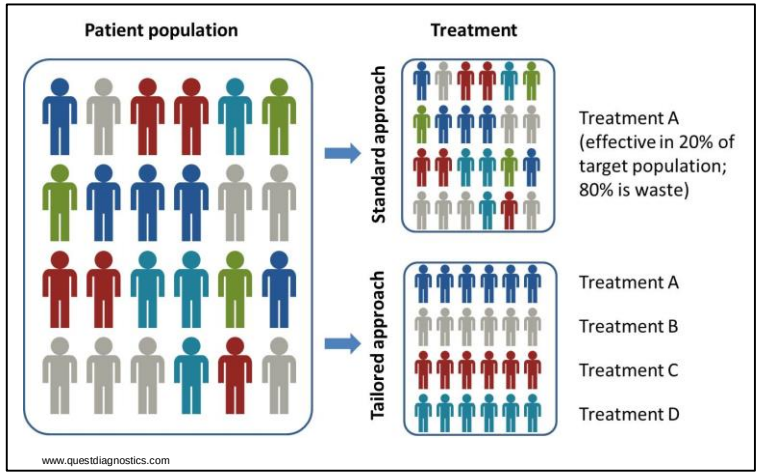
NATURE REVIEWS | DRUG DISCOVERY VOLUME 20 | MAY 2021 | 327

What drives application of Advanced Human Tissue Models in Pharmaceutical Drug Development?

- **Shift in portfolio** from small to complex, engineered molecules that often have multiple targets
- Molecules often **do not cross react with any pre-clinical species** (not even primates)
- **Target(s) & pathway(s)** are not adequately represented in any animal species (i.e. **immune-related**)
- **Additional upcoming Challenges**:
  - *Need for assessing safety & efficacy in children*
  - *Need for assessing safety & efficacy in different ethnicities*
  - *Sometimes small patient population*
  - *Goal to increase benefit/risk, ie strive for more personalized medicine*



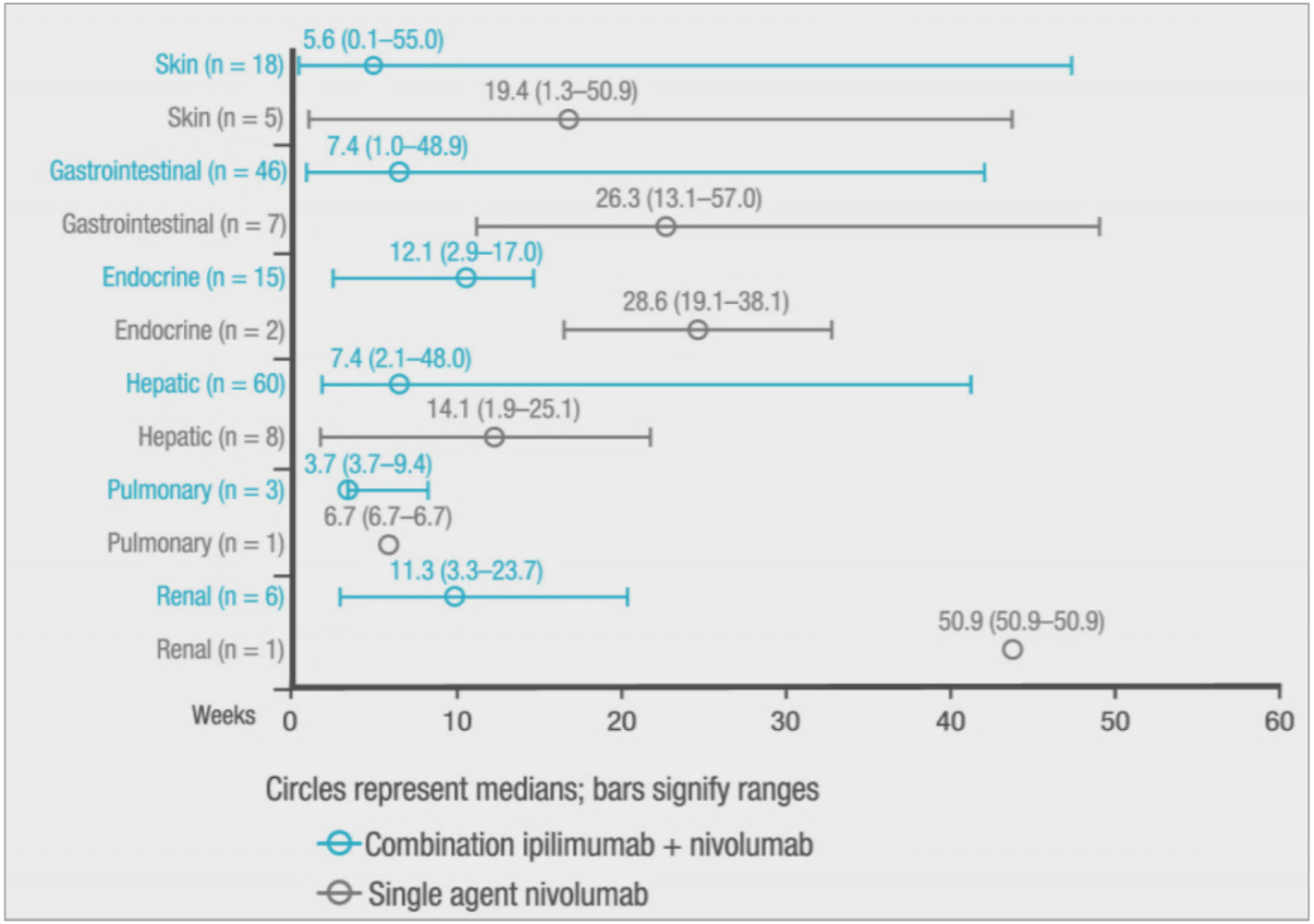




➢ **Conventional pre-clinical *in vivo* testing may not be relevant or simply not possible**

➢ **Urgent need for novel tools to assess the pharmacology & toxicology of these new types of drug candidates**

Cancer Immunotherapy:
Clinical Toxicities of Immune-engaging Antibodies



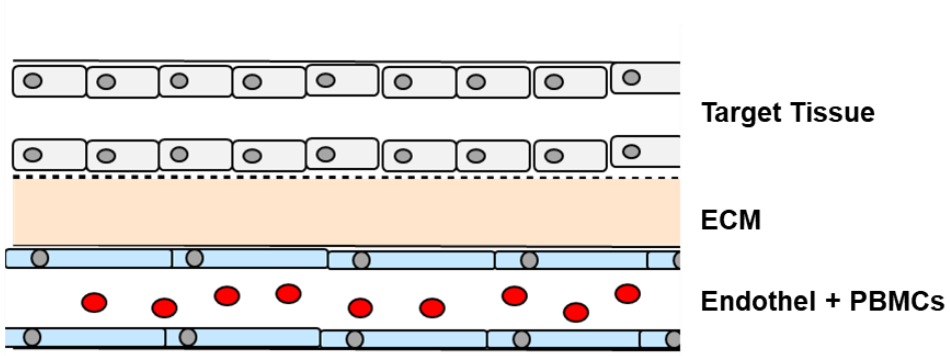
Time to onset of grade 3–4 treatment-related selected adverse events (AEs)

Ipilimumab:Anti-CTLA-4
Nivolumab: Anti-PD-1

Annals of Oncology
Volume 28, Supplement 4, July 2017, Pages iv119-iv142

Suite of Human Models established to address “on-target, off-tumor” safety liabilities of bi-specific antibodies

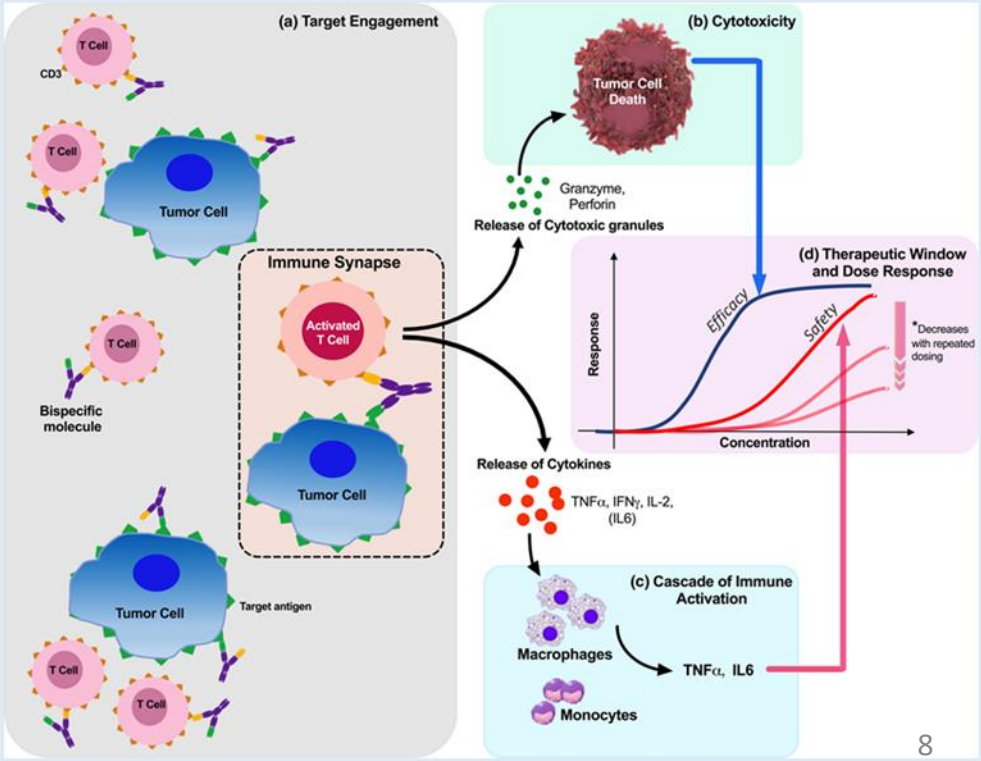
- Primary human tissue, polarized where required
- PBMCs in flow
- All tissue/cell components donor/HLA-matched



Current conventional 2D cell systems lack essential cellular, biochemical, and biophysical factors found in the native organ

Goal to create models that are able to recreate

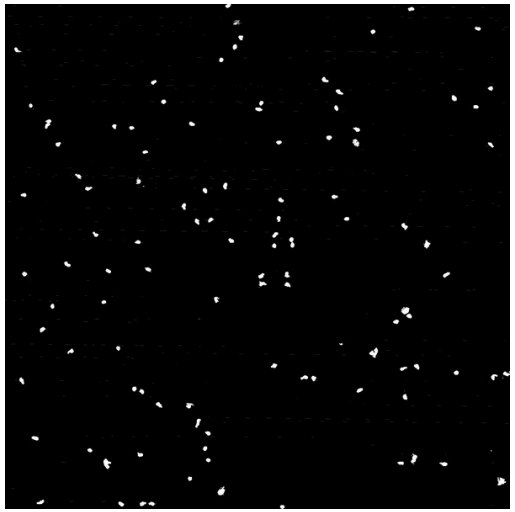
- ***Drug induced activation of immune cells***
- ***Initiation of downstream events including cell migration to site of action***
- ***Measurable effects at site of action that allow drawing conclusions on drug molecule properties, desired & undesired effects, relevance to patient population***



Clinical and Translational Science, Volume: 14, Issue: 1, Pages: 75-85

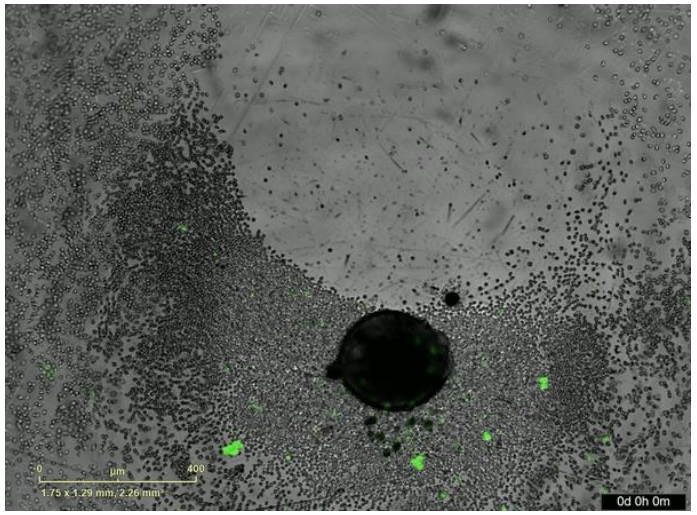
Predict immune-related toxicities early on by detecting key events using advanced human cell systems

Immune cell activation ⇨ **Targeting healthy tissue** ⇨ **Destruction of healthy tissue**



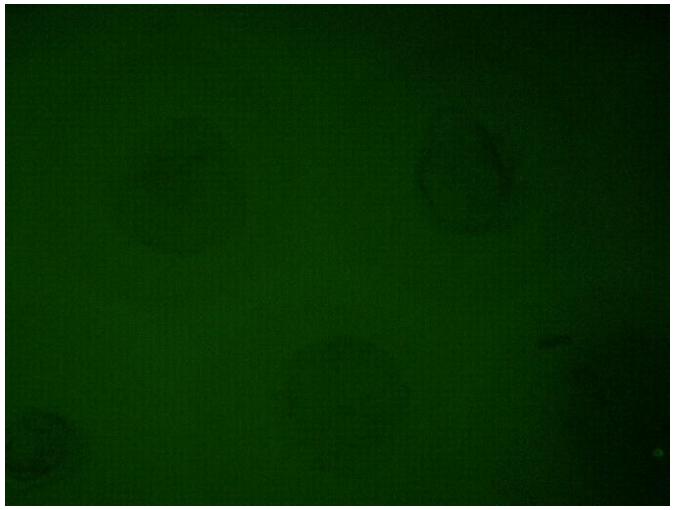
R Villenave

Recruitment of blood leukocytes under flow following TNF-a stimulation



P Godoy/E Breous-Nystrom

Leukocyte infiltration into hepatic spheroids following anti-PD-1 nivolumab treatment



N Gjorevski/M Bscheider

Chemotherapy-induced apoptosis of intestinal organoids

-> Use relevant cell types, readouts & and known relevant positive and negative controls to validate models for specific context of use

Ask the right questions with the right models

From single to multi-organ model approach – focused questions or broad vital functions for de-risking

Cytokine-mediated toxicities

- Rather immediate or short-term effects
- Can be secondary to tumor lysis
- Involve the vasculature and can affect many tissues and organs

On-target / off-tumor toxicities

- Typically restricted to tissues where target is expressed
- Less of a concern if safety window large enough due to significantly higher expression in tumor

Off-target / off-tumor toxicities

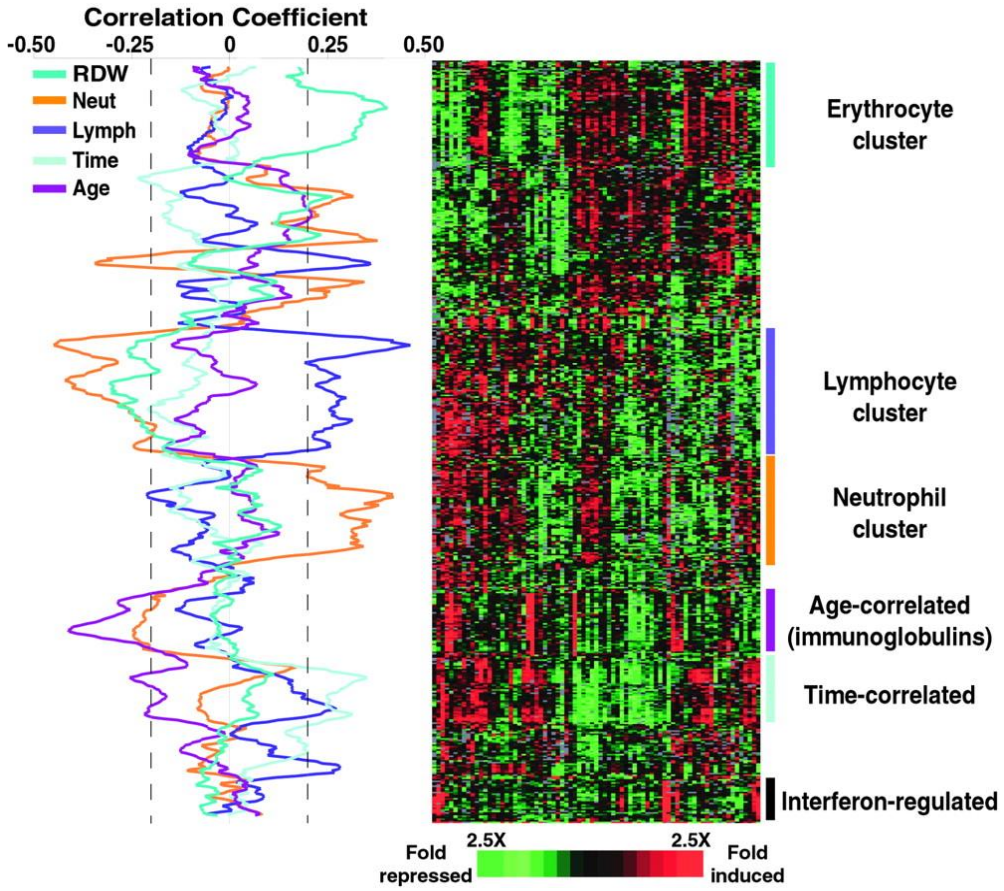
- Difficult to predict as can hit any cell type of the human body
- Can be best addressed with a thorough analysis of off-target peptides

Autoimmunity, infections, inflammation

- Result from an impairment of the immune system
- Can take various forms but could involve similar mode of actions
- Require lymphoid responses

Specifically relevant for T cell engaging molecules and adoptive cell therapies (e.g. Bispecifics and CAR-T)

Example for technical challenges to tackle: Factors affecting blood donor variability



Variation in gene expression patterns in human blood.
Whole blood was drawn from 75 healthy volunteers.

- **Genetic diversity**
- **Environmental factors: immunizations, nutrition, latent infections**
- **Technical factors: Cryopreservation techniques, buffers & media**
- **Sample composition - cellular subsets**

Whitney et al. PNAS 2003

eLife Cancer Biology, Medicine

Human immunocompetent Organ-on-Chip platforms allow safety profiling of tumor-targeted T-cell bispecific antibodies

S Jordan Kerns, Chaitra Belgur, Debora Petropolis, Marianne Kanellias, Riccardo Barrile, Johannes Sam, Tina Weinzierl, Tanja Fauti, Anne Freimoser-Grundschober, Jan Eckmann, Carina Hage, Martina Geiger, Patrick Ray Ng, William Tien-Street, Dimitris V Manatakis, Virginie Micallef, Regine Gerard, Michael Bscheider, Ekaterina Breous-Nystrom, Anneliese Schneider, Anna Maria Giusti, Cristina Bertinetti-Lapatki, Heather Shannon Grant, Adrian B Roth, Geraldine A Hamilton, Thomas Singer, Katia Karalis, Annie Moisan, Peter Bruenker, Christian Klein, Marina Bacac, Nikolce Gjorevski ✉, Lauriane Cabon ✉

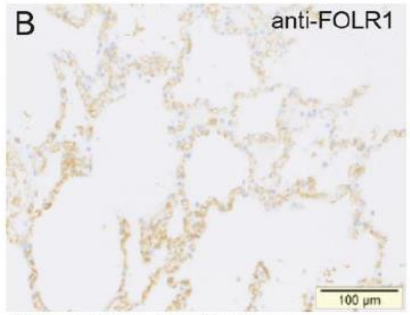
Emulate Inc, United States; Emulate Inc., United States; Roche pRED, Switzerland; Roche pRED, Germany

Research Article · Aug 11, 2021

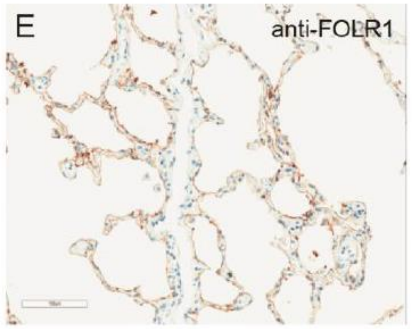
Example: TCB targeting CD3 & FOLR1

Pre-clinical Safety Assessment:

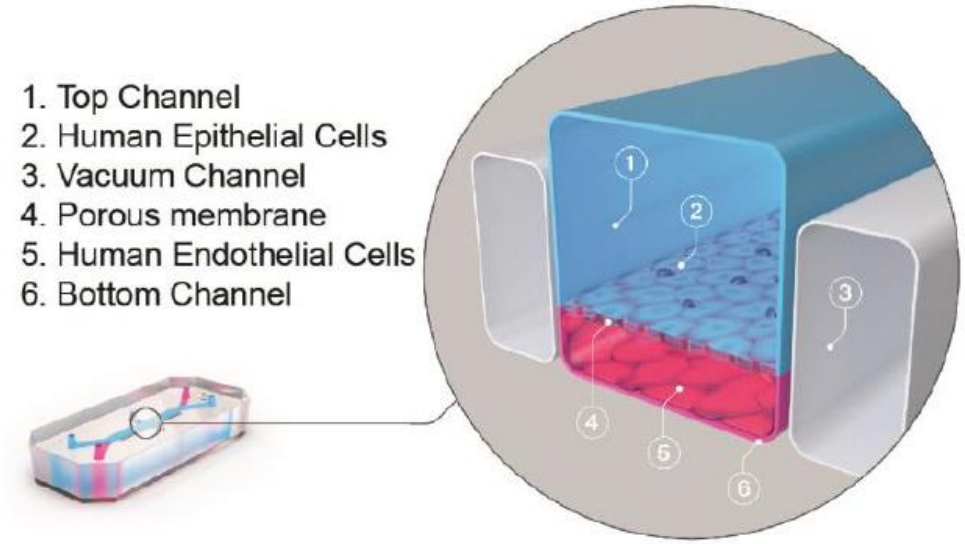
- **efficacious in human breast cancer patient-derived xenograft models**
- **severe on-target toxicity in the lung of cynomolgus monkey**
  - *leukocytic infiltrates in lung tissue indicative of immune mediated toxicity*



Cynomolgus, healthy lung



Human, healthy lung

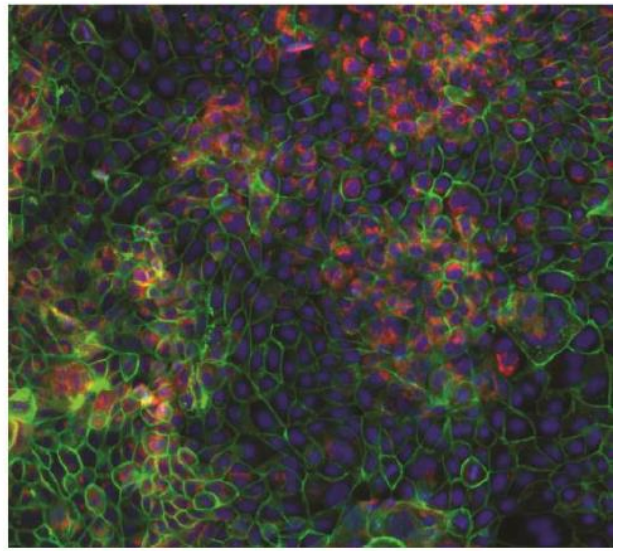


emulate

FOLR1

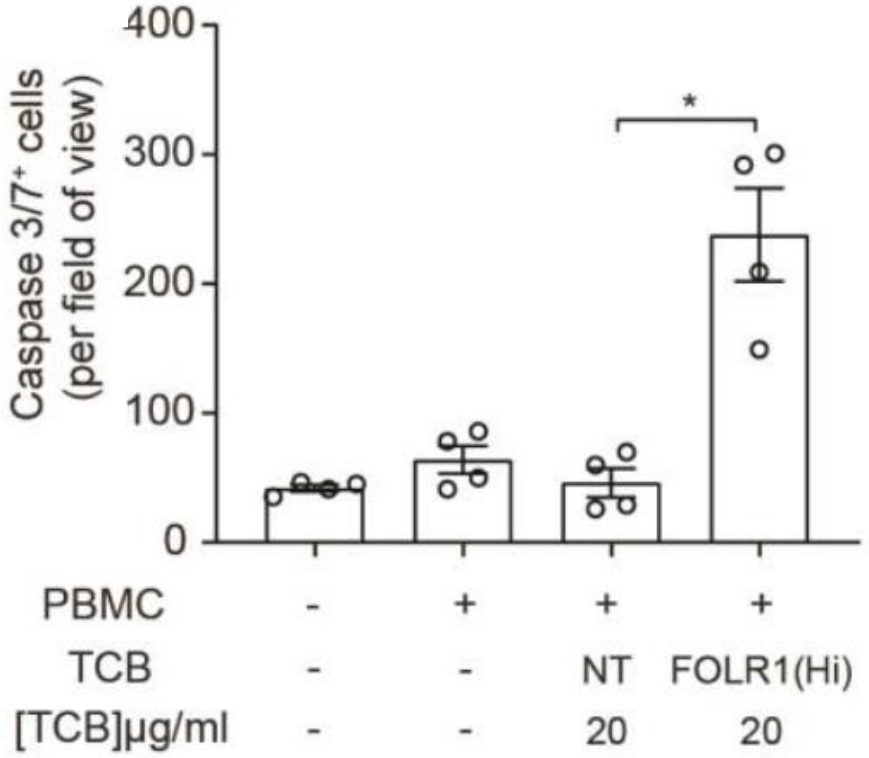
Nucleus

E-cadherin

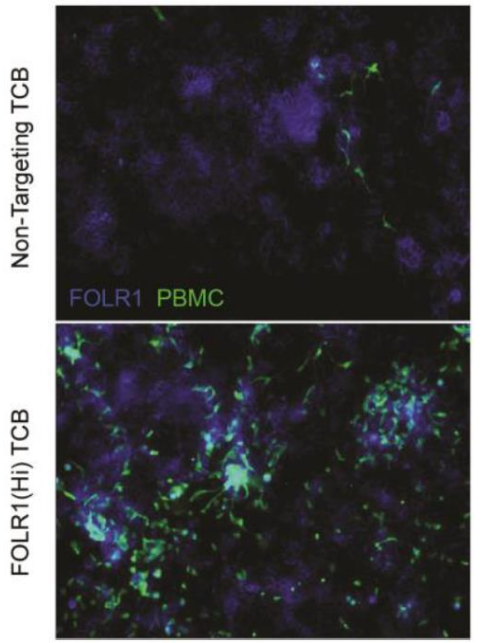


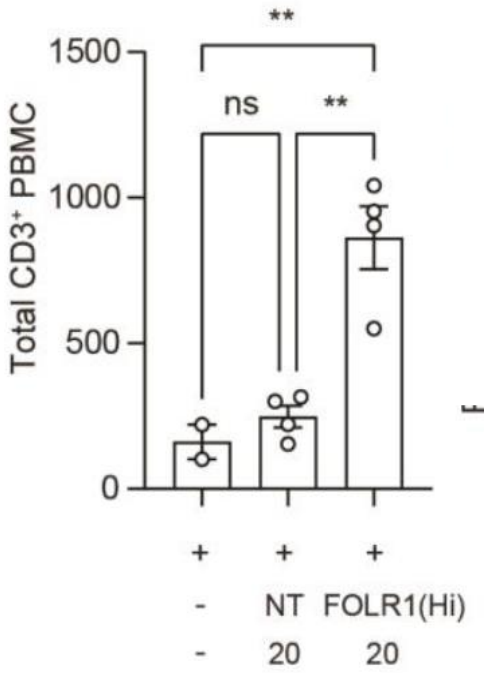
Example: TCB targeting CD3 & FOLR1

Apoptotic cells collected on live chips



PBMC accumulation at sites of target expression consist primarily of CD3-T cells

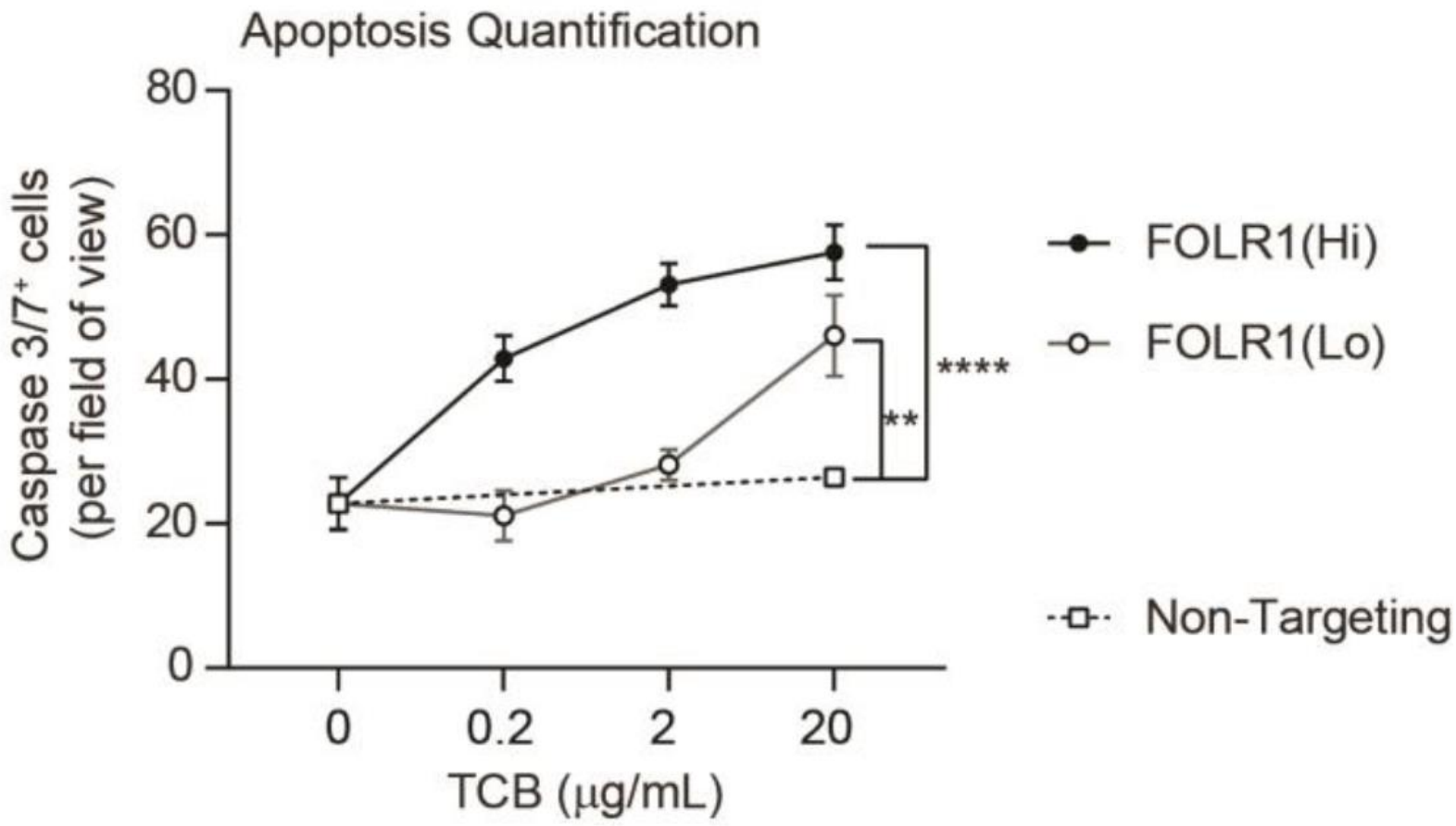




PBMC	*Peripheral Blood Mononuclear Cells*
TCB NT	*Non Targeting TCB*
TCB FOLR1(HI)	*FOLR1-targeting TCB*

Example: TCB targeting CD3 & FOLR1

Model leveraged to identify a safer molecule



Lab on a Chip

ROYAL SOCIETY OF CHEMISTRY

PAPER

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Cite this: *Lab Chip*, 2020, **20**, 3365

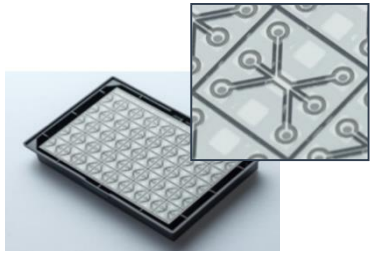
Neutrophilic infiltration in organ-on-a-chip model of tissue inflammation†

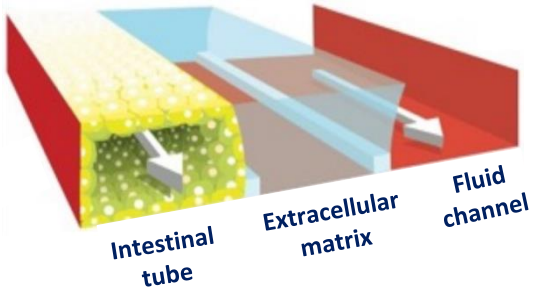
Nikolce Gjorevski, * Blandine Avignon, Régine Gérard, Lauriane Cabon, Adrian B. Roth, Michael Bscheider and Annie Moisan *

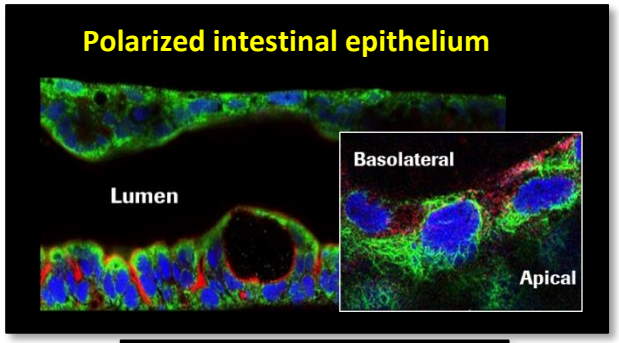
Neutrophilic infiltration in a gut-on-a-chip model of intestinal inflammation

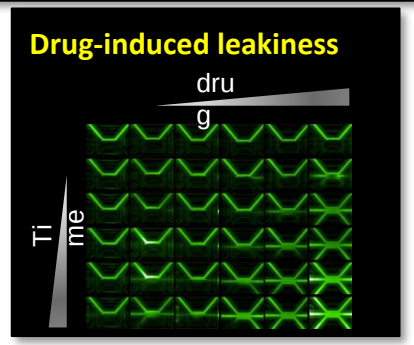
https://www.mimetas.com/en/3d-cell-migration/

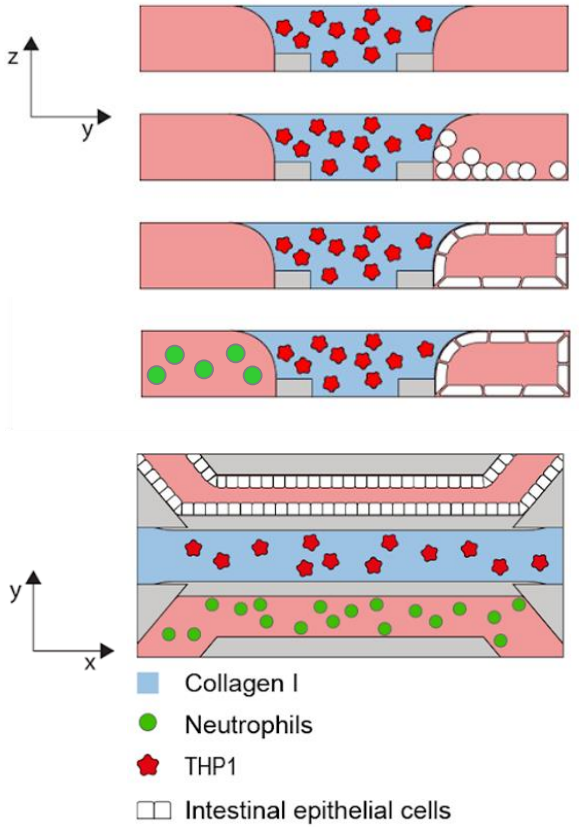
40 x microfluidics chambers
3D cell culture

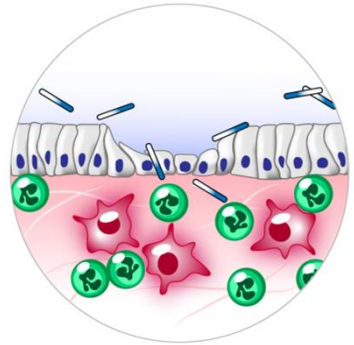




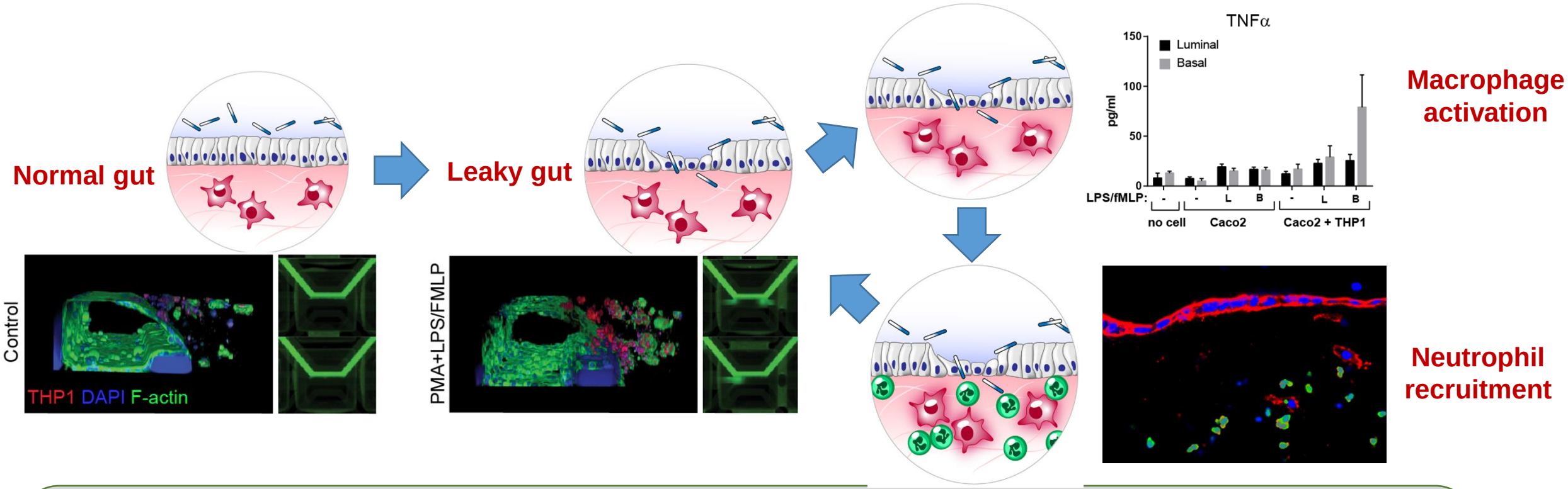








Neutrophilic infiltration in a gut-on-a-chip model of intestinal inflammation



- Tri-culture of epithelial, resident and infiltrating immune cells, capturing their functions and functional interactions
- Infiltrating neutrophils exacerbate the inflammatory process, leading to epithelial damage
- CaCo-based; Missing stromal and vascular cells
- Adaptive immune cell contribution to inflammation not captured

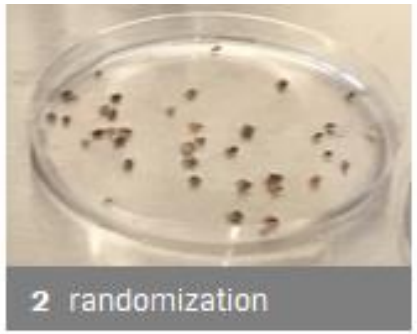
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

Tumor-targeted 4-1BB agonists for combination with T cell bispecific antibodies as off-the-shelf therapy

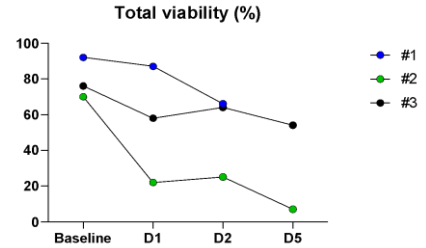
Christina Claus[1]*, Claudia Ferrara[1]*, Wei Xu[1]*, Johannes Sam[1], Sabine Lang[1], Franziska Uhlenbrock[2], Rosmarie Albrecht[1], Sylvia Herter[1], Ramona Schlenker[1], Tamara Hüsser[1], Sarah Diggelmann[1], John Challier[1], Ekkehard Mössner[1], Ralf J. Hosse[1], Thomas Hofer[1], Peter Brünker[1], Catherine Joseph[3], Jörg Benz[3], Philippe Ringler[4], Henning Stahlberg[4], Matthias Lauer[3], Mario Perro[1], Stanford Chen[1], Christine Küttel[1], Preethi L. Bhavani Mohan[1], Valeria Nicolini[1], Martina Carola Birk[1], Amandine Ongaro[1], Christophe Prince[1], Reto Gianotti[1], Gregory Dugan[5], Christopher T. Whitlow[5], Kiran Kumar Solingapuram Sai[5], David L. Caudell[5], Armando G. Burgos-Rodriguez[6], J. Mark Cline[5], Michael Hettich[3], Maurizio Ceppi[3], Anna Maria Giusti[3], Flavio Crameri[3], Wouter Driessen[3], Peter N. Morcos[7], Anne Freimoser-Grundschober[1], Victor Levitsky[1], Maria Amann[1], Sandra Grau-Richards[1], Thomas von Hirschheydt[8], Stella Tournaviti[8], Michael Mølhøj[8], Tanja Fauti[1], Viola Heinzelmann-Schwarz[9], Volker Teichgräber[1], Sara Colombetti[1], Marina Bacac[1], Alfred Zippelius[2], Christian Klein[1], Pablo Umaña[1†]

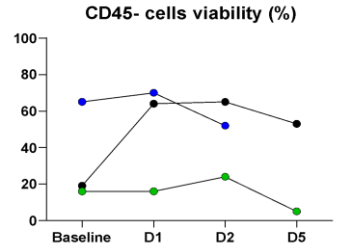
3D tumor explant culture system

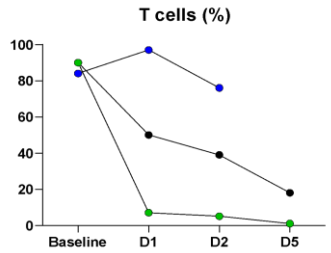


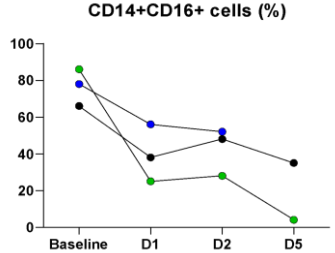
U-CUP (Cellec Biotek)

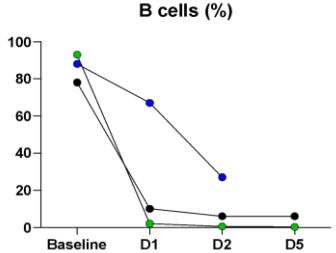
- Viability is **stable for two days** (48h), drops afterwards
- “Viability drop” equally observed in all cell populations









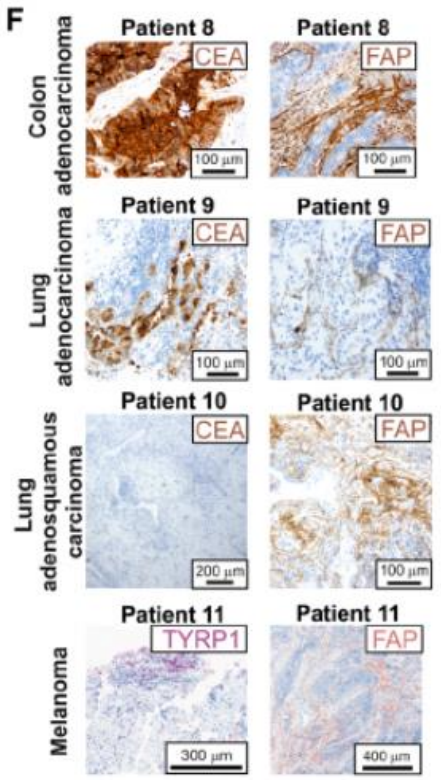


Sylvia Herter, Shamin Li, Tamara Hüsser, Inês Silva and Steffen Dettling

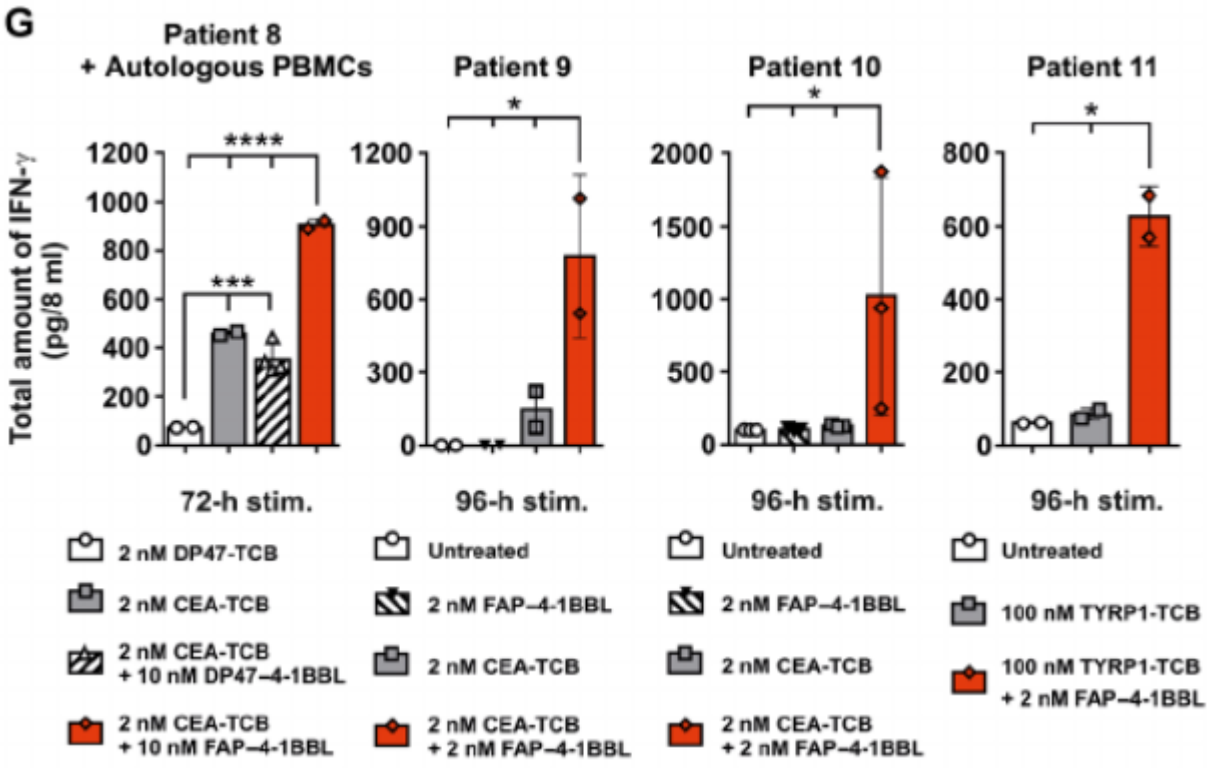
TCB combination with tumor-targeted 4-1BBL agonist

Is natural expression of FAP in human tumor tissues sufficient to provide functional cross-linking for TCBs ?

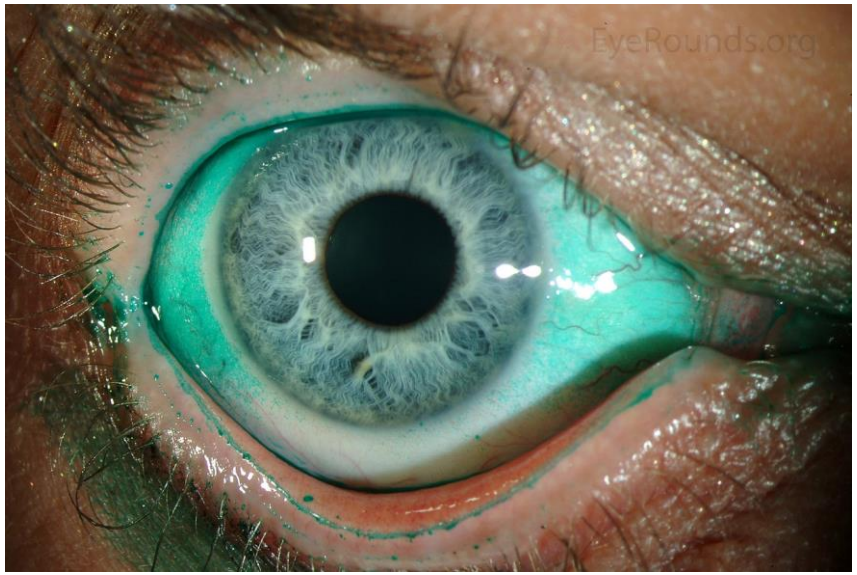
Target (FAP, CEA, or TYRP1) expression in tumor tissue samples



Drug treatment of cultured tumors in 3D system

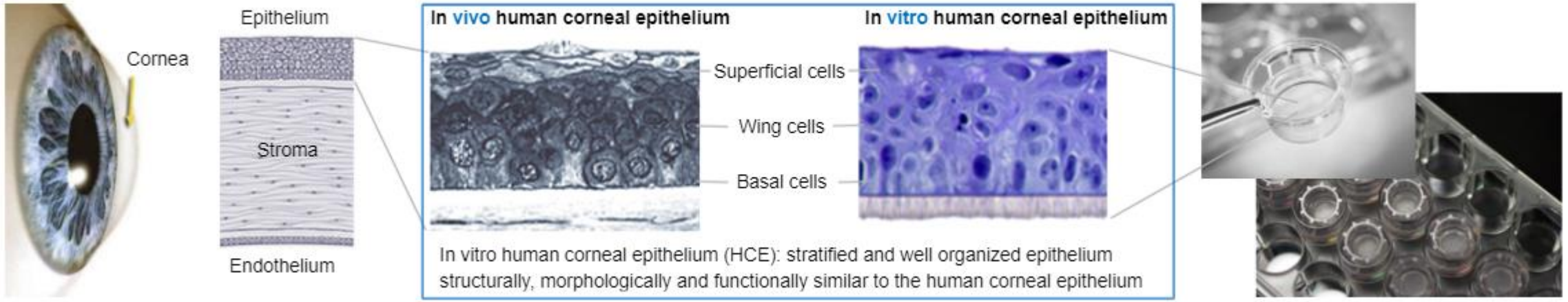


From an OECD qualified test for eye irritation to safety and efficacy modelling for pharmaceutical drugs



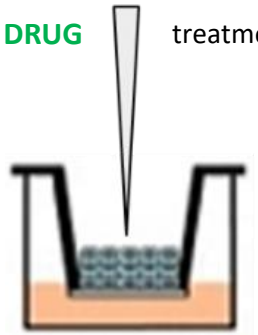
(Karen Dernick, Claudia Korn, Michael Keller, Christian Bucherer, Christoph Ullmer and Stefan Kustermann)

Human 3D Corneal Epithelium as in vitro Model for "Front of the Eye"- Applications and Eye Irritation Tests



Standard condition for eye-irritation test

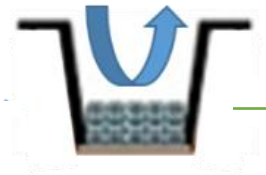
DRUG treatment topical (30 ul)

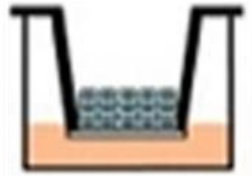


300 ul medium

0,5 - 4 hours incubation

wash insert (2 x 10 ml PBS)

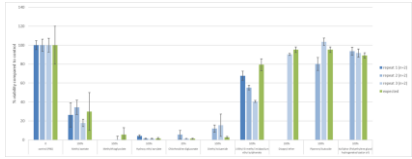




300 ul medium

Readouts:

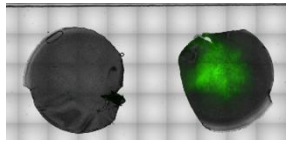
1) OECD Test for eye irritation



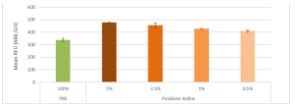
Cell viability – MTT assay

and/or:

2) Translational imaging readout

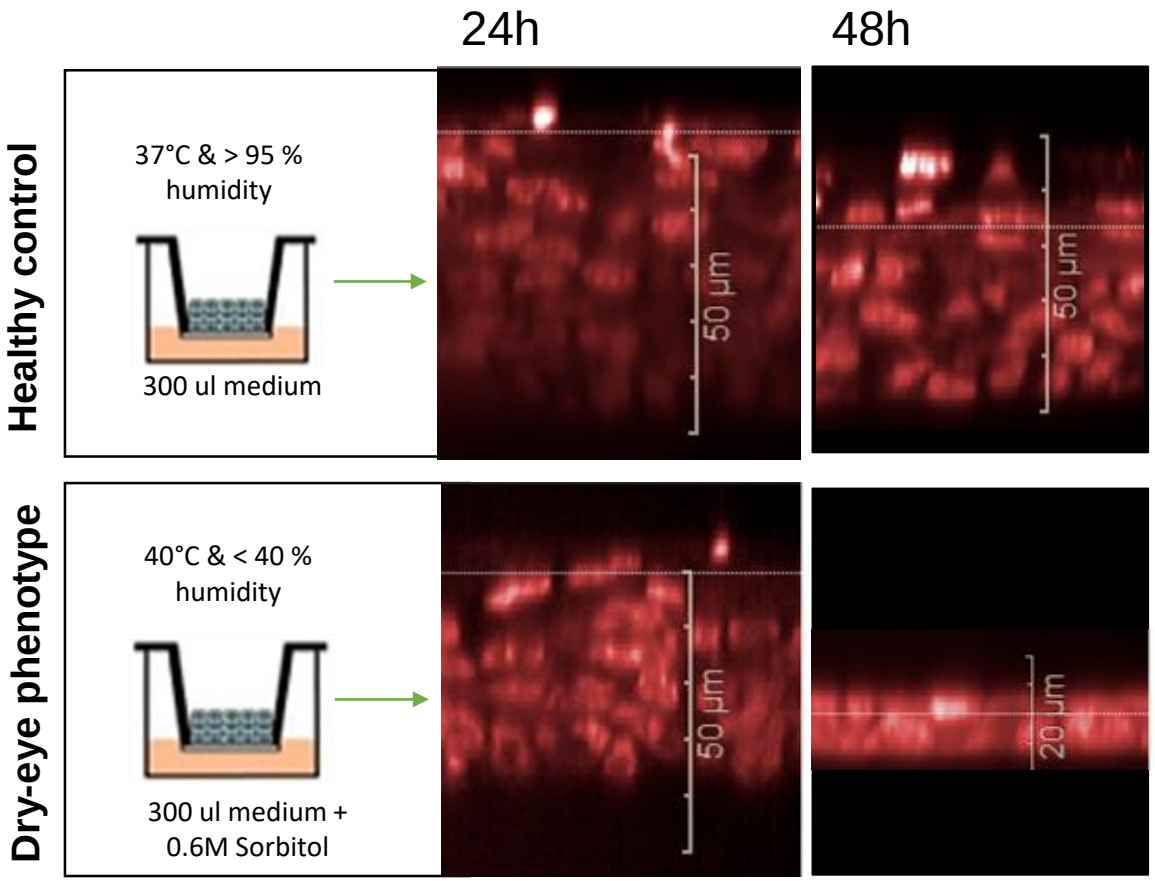


control 5% PVD-I

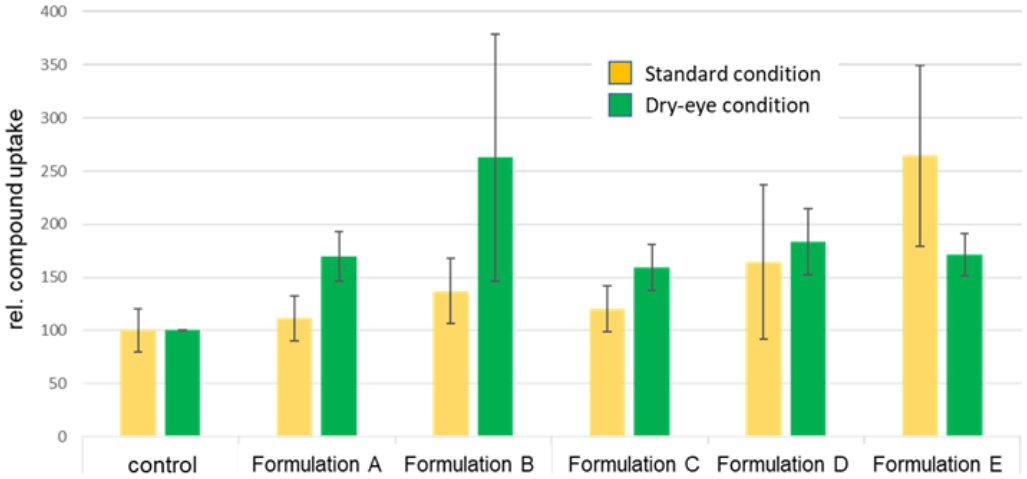


Oregon-green-dextran quantification as translation endpoint to patients

Improved prediction of efficacy using «dry eye disease model»: disease phenotype and formulation drives efficacy



4h topical treatment

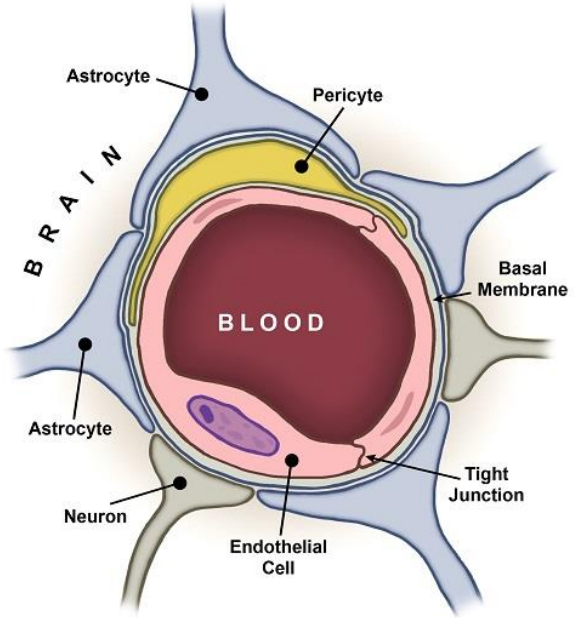


- Formulation-type is impacting efficacy
- Dry-eye condition shows increased efficacy after overnight incubation
  - Proposed mechanism: higher uptake of compound due to impaired barrier

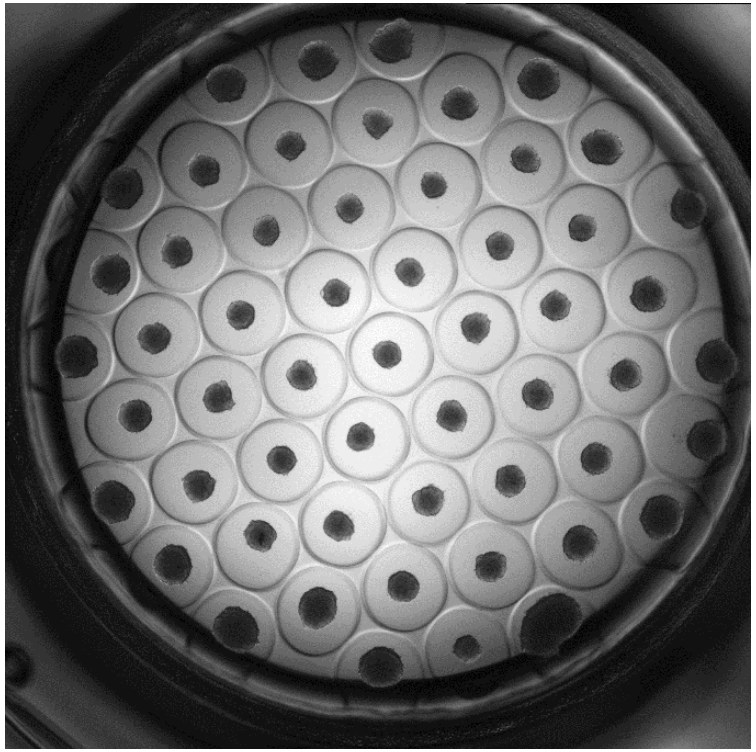
- **Dry-eye phenotype can be induced in the model and leads to a decrease in the epithelial thickness after 48h**

A Blood Brain Barrier Model based on Organoids

(Roberto Villasenor Solorio)

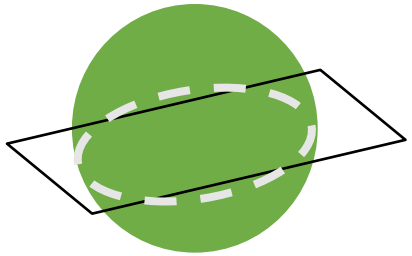


BBB organoid arrays for high-throughput screening



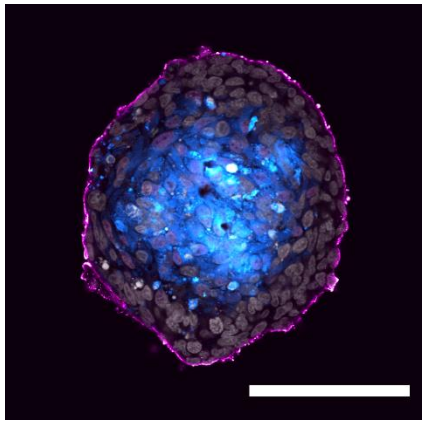
- **Up to 3000 organoids per plate**
- **Highly reproducible size (150 – 250 mm Ø)**
- **Compatible with automated microscopy**

Self-assembly of the human neurovascular unit

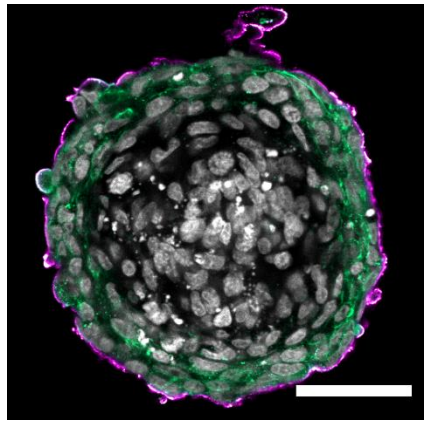


Imaging frame at spheroid core

Endothelial cells Astrocytes



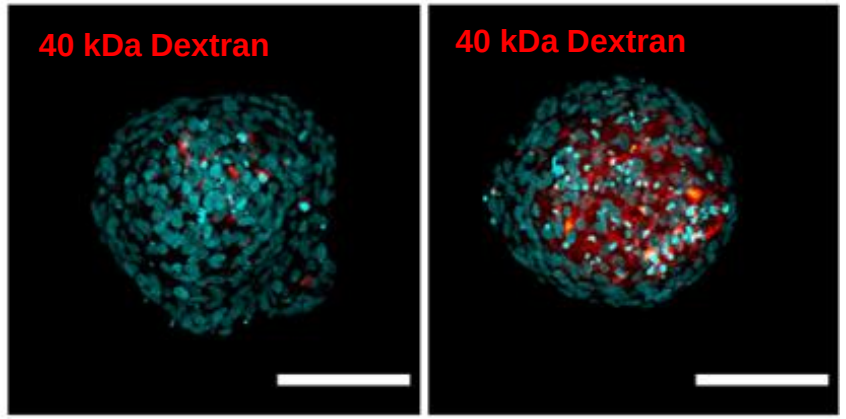
Endothelial cells Pericytes



BBB organoids recapitulate barrier properties for biologics

BBB organoid

Organoid without BBB

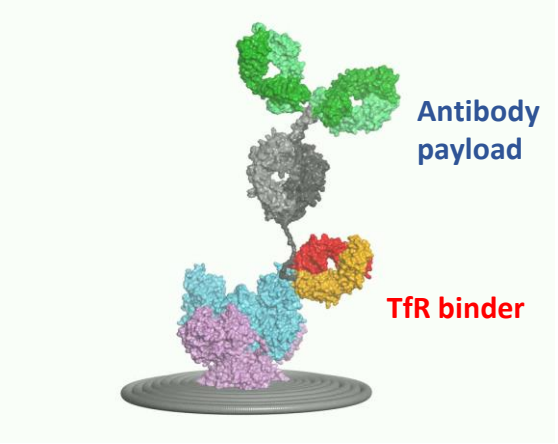


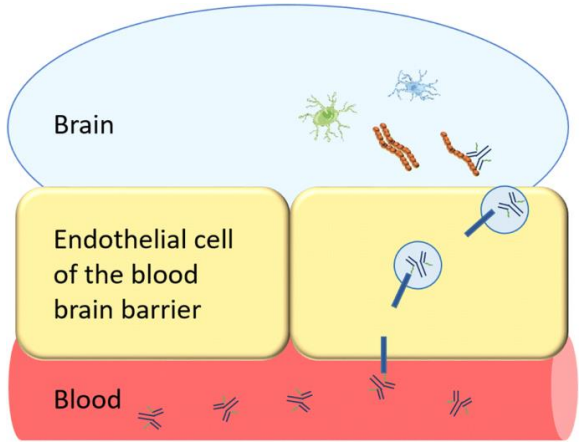
Low Dextran permeability

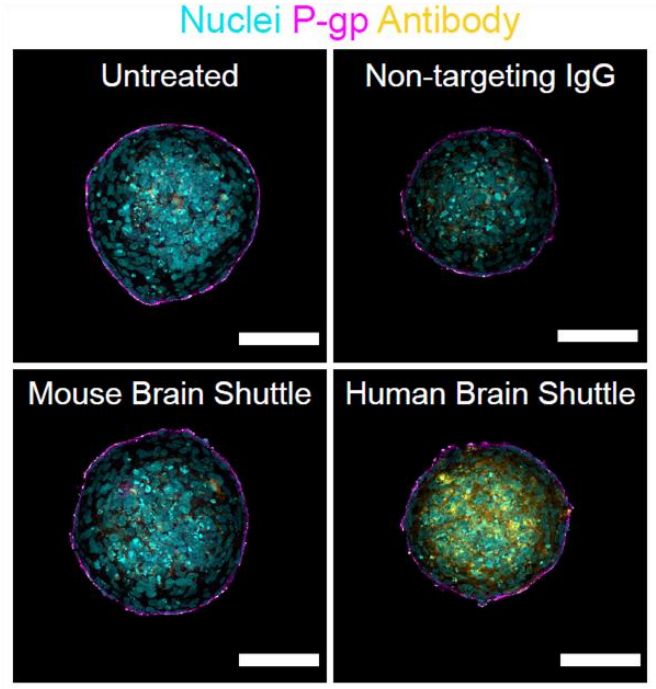
Increased Dextran permeability

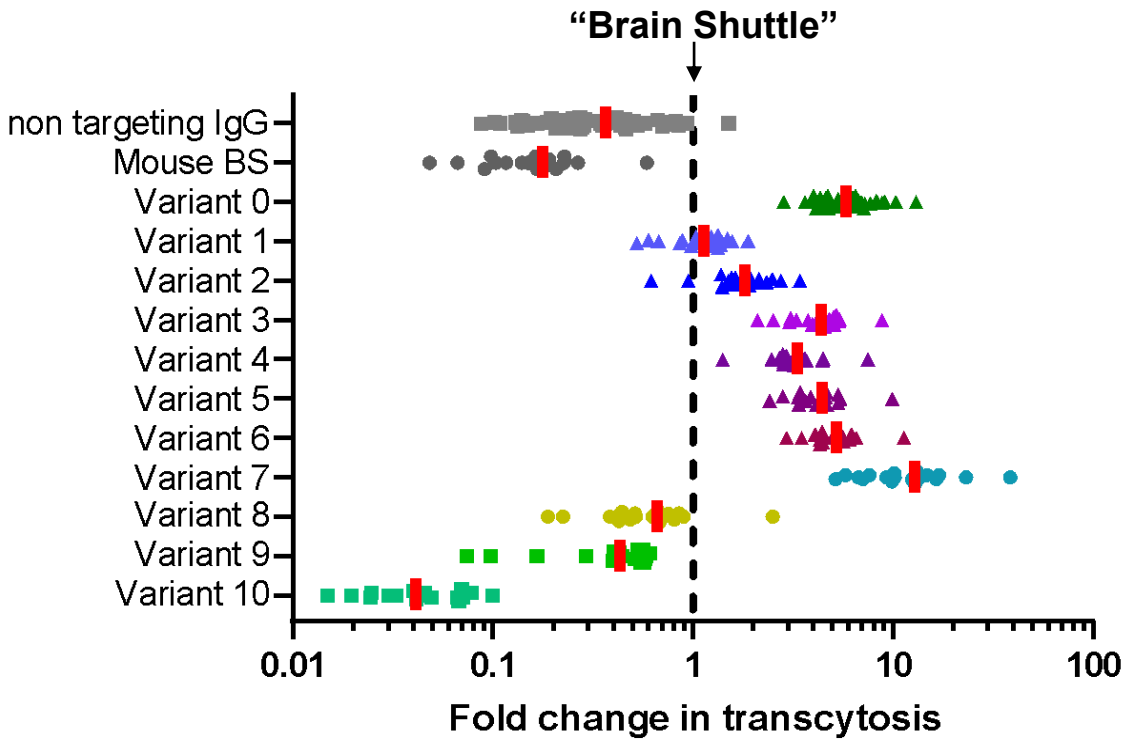
Simonneau, et al. FBCNS, 2021

Screening of new modalities for CNS delivery
BBB organoids accelerate discovery and optimization cycles





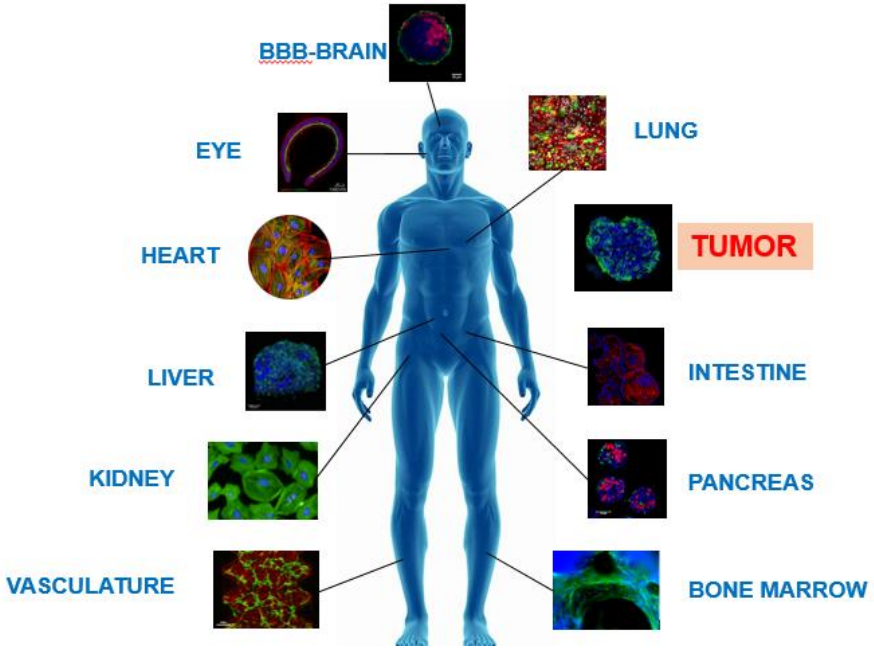


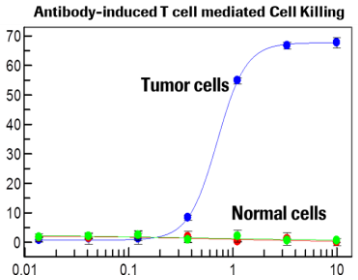


European Journal of Nuclear Medicine and Molecular Imaging volume 46, pages2848–2858 (2019)

Where do we want to go ?

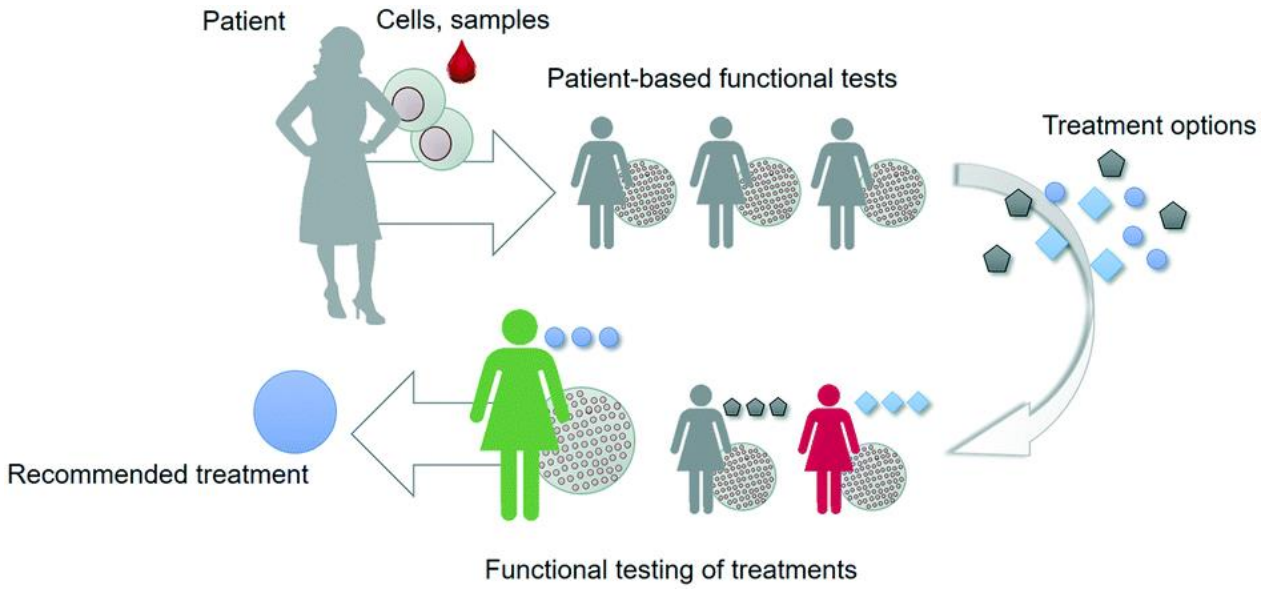
In vitro efficacy & safety assessment for EIH-enabling





«in vitro therapeutic index»

Patient-derived models enabling personalizing clinical trials




PERSPECTIVE

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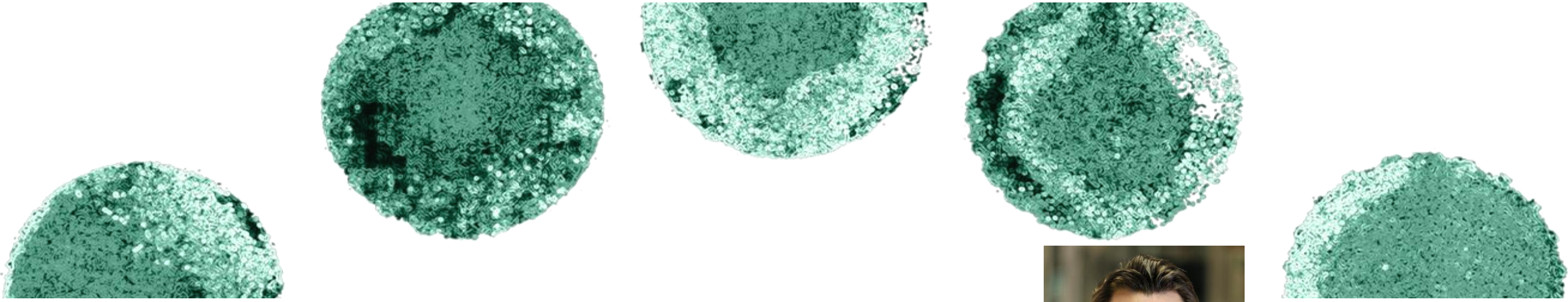
Cite this: *Lab Chip*, 2019, **19**, 198

Personalised organs-on-chips: functional testing for precision medicine

Albert van den Berg,[cd] Christine L. Mummery,[ab] Robert Passier[a] and Andries D. van der Meer[*a]

A strong commitment to build on human cell based approaches to support drug development:

Roche Institute for Translational Bioengineering (ITB)

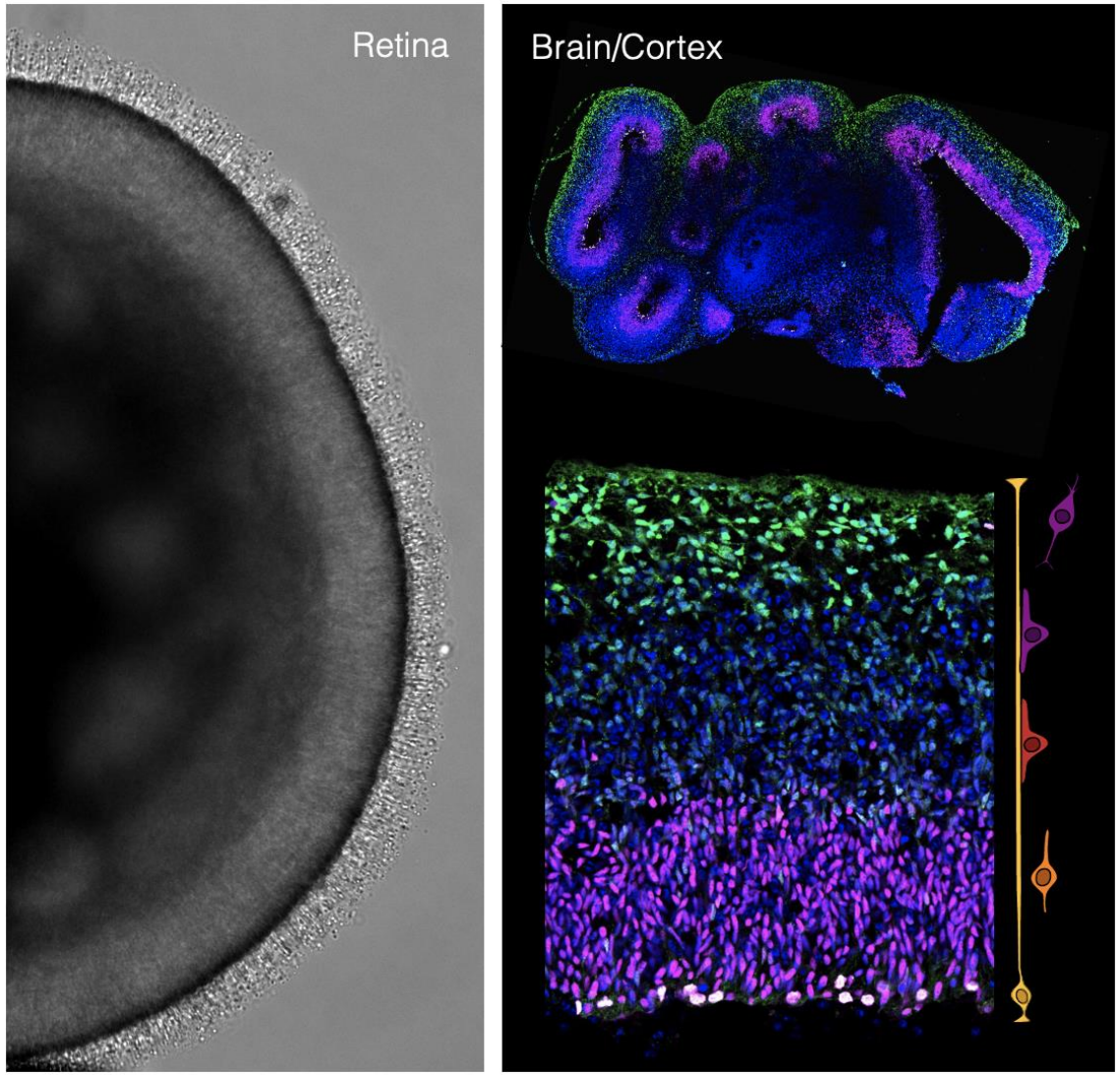


An incubator and catalyst for big, bold ideas and creative organoid technologies that can be translated into drug development for the benefit of patients

Prof M. Lütolf

Roche Institute for
TRANSLATIONAL
BIOENGINEERING

'Organoid Factory' : Brain and retina organoid manufacturing



- **High-throughput, automated mass production** of retinal and brain organoids
- Integrated **quality control** module (imaging- and AI-based) with organoid sorter
- Collaboration with selected external partners on specific aspects (e.g. organoid sorting technology)
- Explore miniaturization and bioreactor-free culture
- Proof-of-principle results for manufacturing of other organoid systems

Advanced Human Tissue Models in Drug Development: Outlook

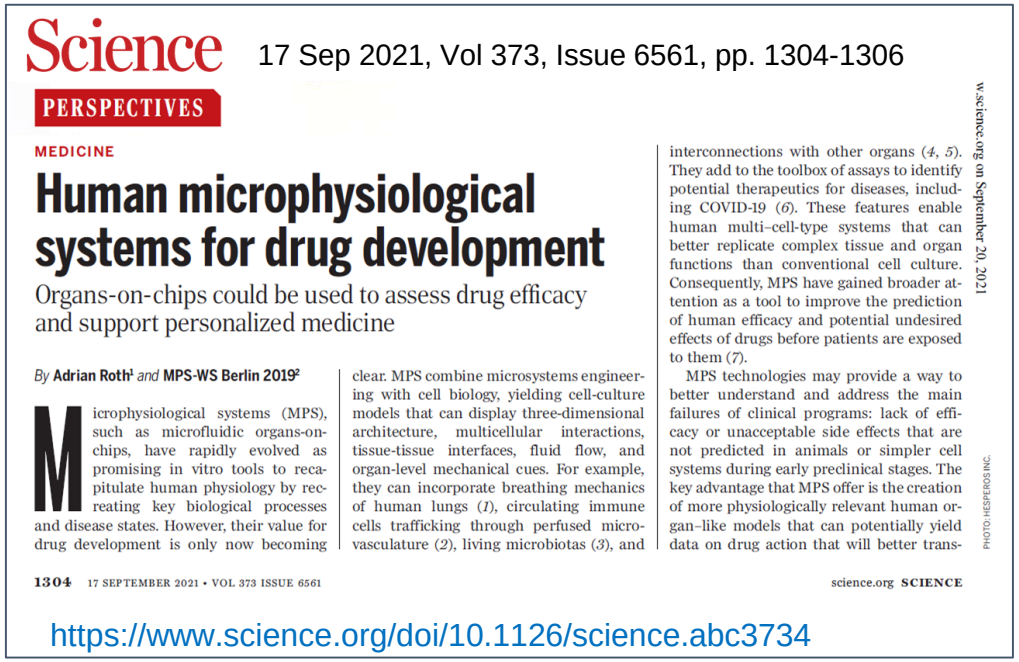
- **Significant Investments in Academia, Biotech & Pharma have led to a series of encouraging Use Cases that demonstrate the Potential of more complex, physiologically relevant human Cell Models**

- **Broad Industry Adoption is low due to**
  - **Immaturity of some of the Systems**
  - **Complex technical Set-up not suited for Scaling and daily Use**
  - **High Investment needed – Pletora of different Approaches**
  - **Biological Relevance not convincingly shown and/or Lack of Superiority over already exisitng Models**

- **Areas with high unmet Need could help driving Application forward, ie where conventional Models are not an Option (e.g. Immunology), where lean Drug Development Paths are possible (e.g. Rare Diseases), where fast Reacting to an urgent Need is warranted (e.g. COVID)**

- **Advanced Human Tissue Models not only can significantly improved pre-clinical Development Phases – they could as well become Game Changers for Clinical Development (i.e. Bedside-Bench-Bedside, Personalized Medicine)**

- **Next to access to Patient Tissue/Cells – Access to Patient Data is key for renewing Drug Development Paradigm**



Science 17 Sep 2021, Vol 373, Issue 6561, pp. 1304-1306

PERSPECTIVES

MEDICINE

Human microphysiological systems for drug development

Organs-on-chips could be used to assess drug efficacy and support personalized medicine

By Adrian Roth[1] and MPS-WS Berlin 2019[2]

Microphysiological systems (MPS), such as microfluidic organs-on-chips, have rapidly evolved as promising in vitro tools to recapitulate human physiology by recreating key biological processes and disease states. However, their value for drug development is only now becoming clear. MPS combine microsystems engineering with cell biology, yielding cell-culture models that can display three-dimensional architecture, multicellular interactions, tissue-tissue interfaces, fluid flow, and organ-level mechanical cues. For example, they can incorporate breathing mechanics of human lungs (*1*), circulating immune cells trafficking through perfused microvasculature (*2*), living microbiotas (*3*), and interconnections with other organs (*4*, *5*). They add to the toolbox of assays to identify potential therapeutics for diseases, including COVID-19 (*6*). These features enable human multi–cell-type systems that can better replicate complex tissue and organ functions than conventional cell culture. Consequently, MPS have gained broader attention as a tool to improve the prediction of human efficacy and potential undesired effects of drugs before patients are exposed to them (*7*).

MPS technologies may provide a way to better understand and address the main failures of clinical programs: lack of efficacy or unacceptable side effects that are not predicted in animals or simpler cell systems during early preclinical stages. The key advantage that MPS offer is the creation of more physiologically relevant human organ–like models that can potentially yield data on drug action that will better trans-

1304 17 SEPTEMBER 2021 • VOL 373 ISSUE 6561 science.org SCIENCE

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www.science.org on September 20, 2021

https://www.science.org/doi/10.1126/science.abc3734

Stem Cell Reports ISSCR

Commentary OPEN ACCESS

Challenging the pipeline

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[2]NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany
[3]3R-Center for *In vitro* Models and Alternatives to Animal Testing, Eberhard Karls University Tübingen, Tübingen, Germany
[4]Translational Safety Sciences, Theravance Biopharma US, Inc., South San Francisco, CA, USA
[5]Personalized Healthcare Safety Interface, Product Development Safety, Roche Innovation Centre Basel, Basel, Switzerland
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