Pathology readouts of complex in vitro models in safety assessments

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New Approach Methodologies (NAMs)

Definition

Humanized animals or genetically altered organisms

Simple & complex in vitro systems

Computational methods

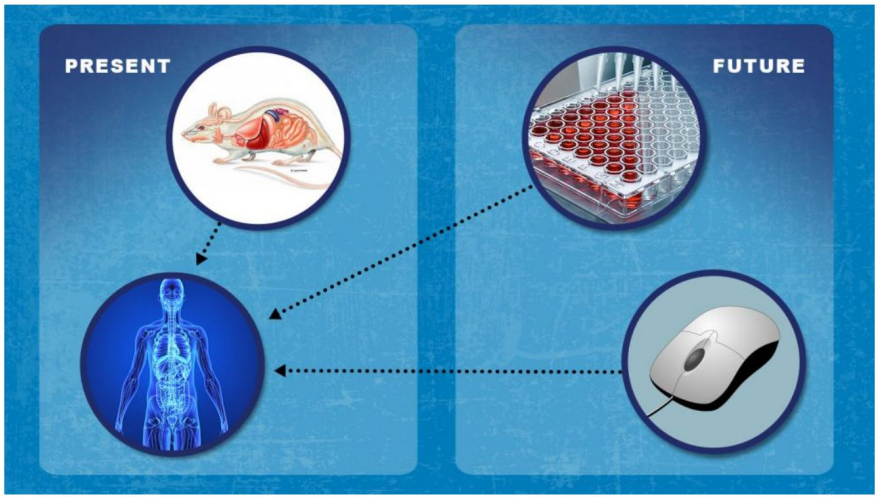
*"CDER considers NAMs to include a broad range of methods such as **in vitro, in chemico,** and **in silico** methods. **In vivo** methods can also be considered NAMs when they **improve predictivity,** shift studies to **phylogenetically lower animals**, or otherwise help replace, reduce, and refine animals use (i.e. **3Rs**) in development programs"* [1]

NAMs are defined as any **technology**, **methodology, approach**, or combination that can provide information on chemical hazard and risk assessment **without the use of animals**, including **in silico**, **in chemico**, **in vitro**, and **ex vivo** approaches (ECHA, 2016b; EPA, 2018d) [2, 3]

New Approach Methodologies (NAMs)

Definition

NAMs are not necessarily newly developed methods; rather, it is their **application** to each agency's regulatory decision-making process or **replacement of a traditional testing requirement** that is new.[4]

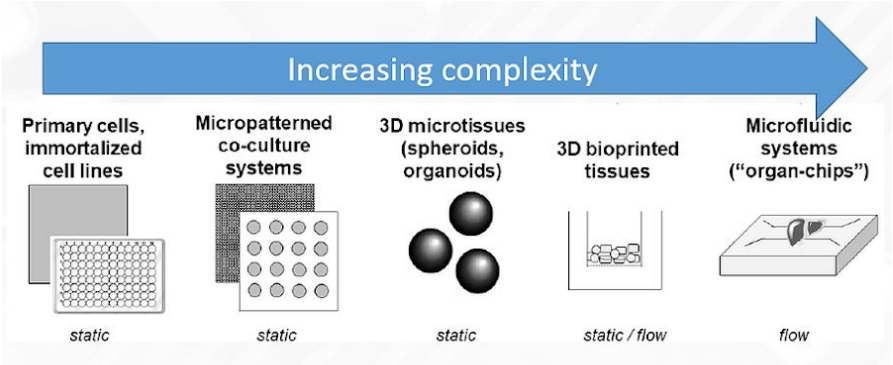


Advancing Alternative Methods at FDA [6]

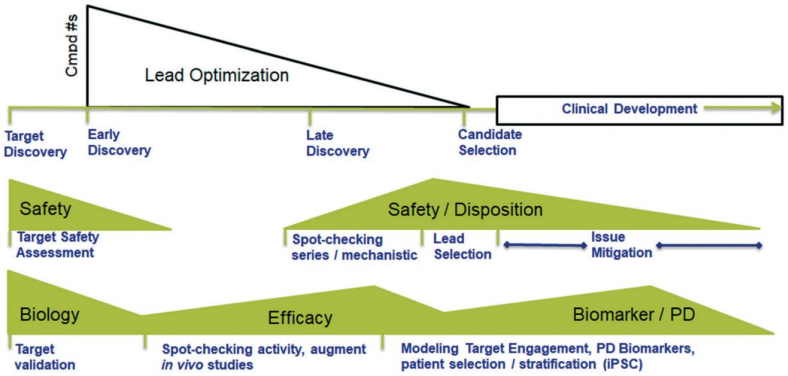
Complex in vitro models (CIVM)

Definition and potential applications in drug development

What are Complex in vitro models[7]



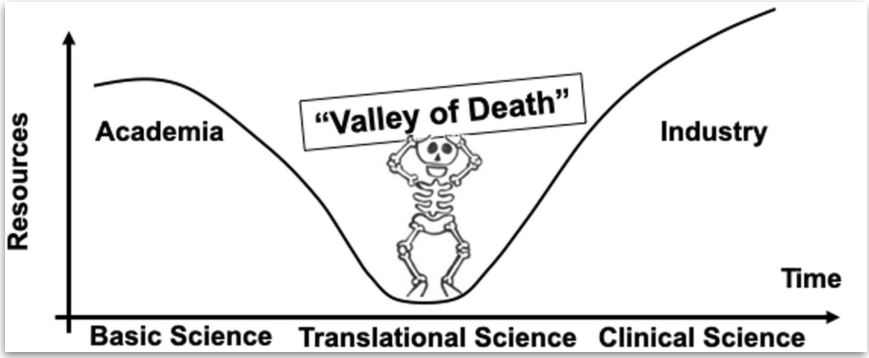
Opportunities in drug discovery/development process with potential CIVM applications[8]



New Approach Methodologies (NAMs)

Why NAMs & CIVM?

Given the limitations associated with animal testing → need for faster, less expensive, and more informative and more predictive new approaches to gathering toxicological information



Compound attrition rates remain challenging for drug companies [8]

- *Bioethical, Reproducibility, and Translational Challenges of Animal Models* [9]
- *low animal-to-human translational success rates - "translational failure"* [10, 11]

Strong push to **move away from NHP** testing

- *Cynomolgus monkey as "**endangered species**"*
- ***Supply constraints**, **Illegal trafficking** of monkeys for laboratory use, occasionally **challenges on health status** of monkeys* [12-20]

New Approach Methodologies (NAMs)

Regulatory landscape

- Regulatory Laws, Guidances (e.g. ICH, EMA, FDA..) already incorporated the use of NAMs FDA Modernization Act 2.0, FDA Modernization Act 3.0 introduced
- Rapid evolution of new technologies out-pacing ability to integrate new tools into current testing framework(s)
- Literature on framework for regulatory acceptance [22], increasing confidence in NAMs, standards and best practices [23, 24] etc. available
- Collaborative efforts necessary between generators of these methods, users, regulators (end users)
- Dialogue and education between scientists and regulators Consortia & Initiatives

FDA Modernization Act 2.0

"Allows for alternatives to animal testing for purposes of drug and biological product application"

23rd December 2022 – U.S. House Approval

Republicans and Democrats Introduce the FDA Modernization Act 3.0, Requiring FDA to Implement Animal Testing Reforms Passed by Congress Over a Year Ago

Regulatory Guidances

NAMs relevant ICH guidelines

Regulatory Guidances

ICH S10 Photosafety Evaluation of Pharmaceuticals Guidance for Industry, 2015 – Use of in chemico and in vitro approaches to assess phototoxicity potential.

ICH S5(R3) Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals Guidance for Industry, 2021 – Description of testing strategies utilizing alternative assays for the assessment of malformations and embryofetal lethality, and the qualification process for these alternative assays.

ICH M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals To Limit Potential Carcinogenic Risk Guidance for Industry, 2018 – Use of computational approaches for the assessment of mutagenic potential of drug impurities.

ICH S3A Guidance: Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies: Focus on Microsampling Questions and Answers Guidance for Industry, 2018 – Use of microsampling in toxicity studies.

Oncology Pharmaceuticals: Reproductive Toxicity Testing and Labeling Recommendations Guidance for Industry, 2019 – Potential use of alternative assays, such as fit-for-purpose in vitro or ex vivo, or nonmammalian in vivo assays for assessment of reproductive toxicity.

https://www.fda.gov/science-research/about-science-research-fda/regulatory-guidances-list-alternative-methods

NAMs Consortia, Initiatives & Workshops

A way to interact (formally/informally) with regulators & colleagues across industries

NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Link to NC3Rs supported projects

IQ MPS

IQ Microphysiological Systems Affiliate

FDA: Desire to develop more predictive models of toxicological response:

- Tox21 Consortium,
- FDA Predictive Toxicology Roadmap,
- FDA Alternative Methods Working Group

FDA's Alternative Methods Working Group

ToxMinds

THINKING AHEAD IN TOXICOLOGY

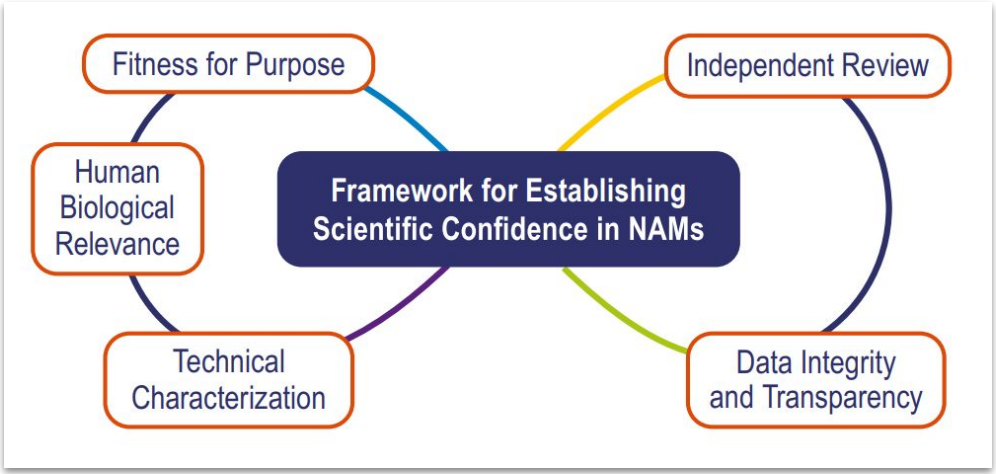
Adopting New Approach Methodologies (NAM) in the next generation risk assessment (NGRA)

https://toxminds.com/adopting-new-approach-methodologies-nam-in-the-next-generation-risk-assessment-ngra/

Framework & Role of Pathologists

Contribution to the scientific confidence in NAMs applied in drug development

A framework for establishing scientific confidence in new approach methodologies [22]

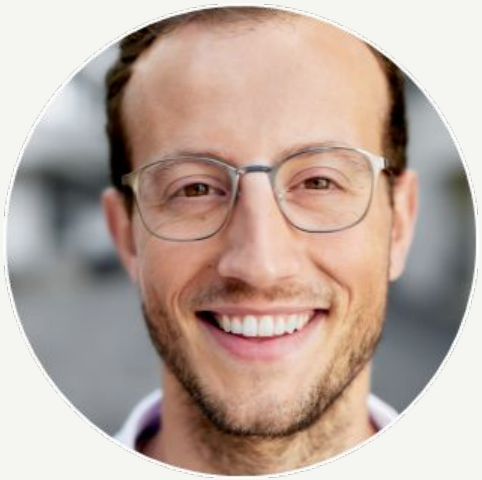


- What role can we play as a pathologist?
- How can we contribute with our expertise and our technologies?
- How to get involved and familiar with CIVMs as a Pathologist?

Pathology readouts of complex in vitro models (CIVM)

Acknowledgement

Luisa Bell, PhD thesis
Blood-brain barrier transport in Alzheimer's disease

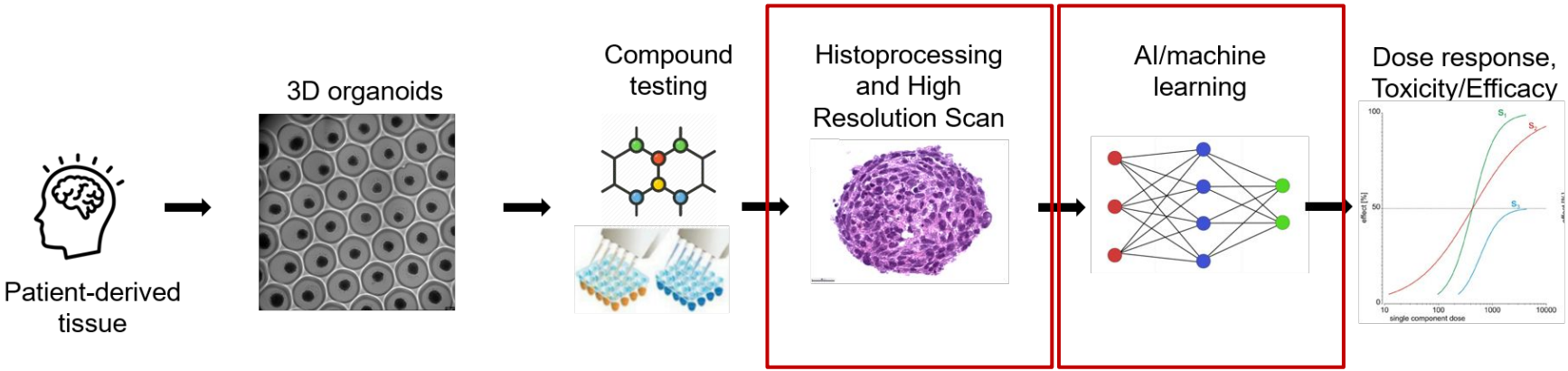


Marius Harter, Master thesis
PBMC co-cultured gut organoids for toxicity evaluation

Pathology readouts of CIVMs

What role can we play as Pathologists?
How can we contribute with our technology and expertise?

High - throughput compound screening (in vitro)



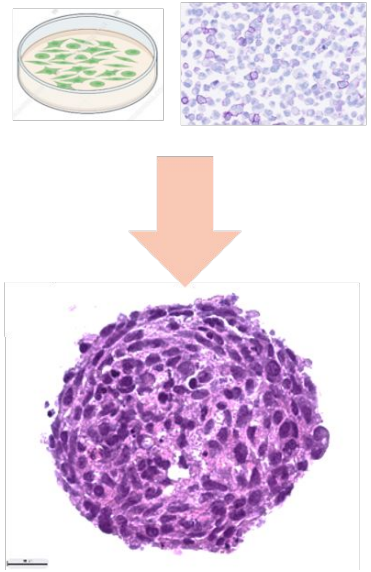
1. Pathology tissue technologies, clinpath & automated (AI-based) readouts
   → high-throughput increase
2. Pathologists expertise guide the selection of the most predictive non-clinical model for a defined context of use

Bell et al., in preparation

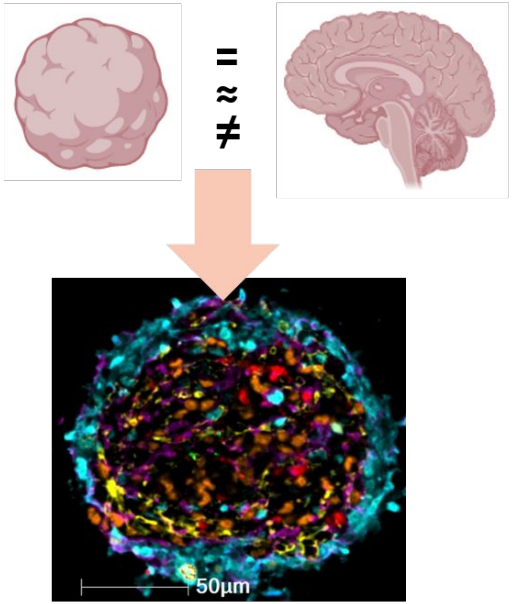
Pathology readouts of CIVMs

How can we contribute with our technology and expertise? Ex. blood-brain barrier (BBB) organoids

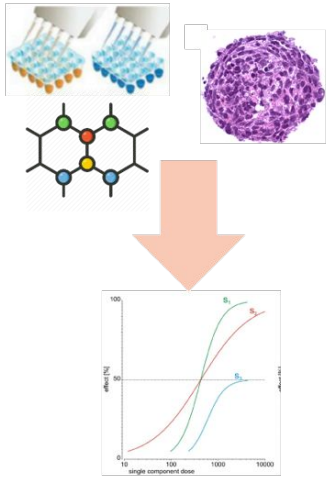
- Molecular pathology-guided model engineering



- Model characterization and validation



- Functional readouts for efficacy and toxicity
  - Compound distribution
  - Toxicity assessment

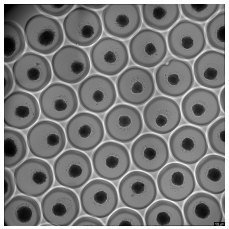


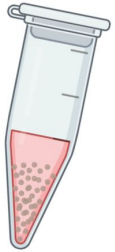
Bell et al., in preparation

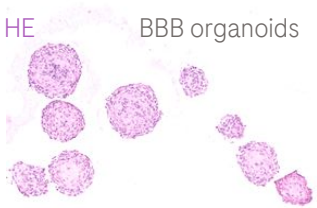
Automated AI-guided histology readouts of complex in vitro models

How to get involved with CIVM → Workflow (Ex. blood-brain barrier (BBB) organoids)

Wet lab

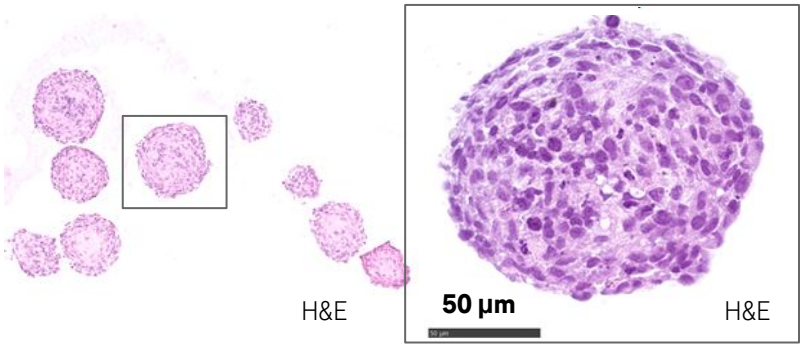






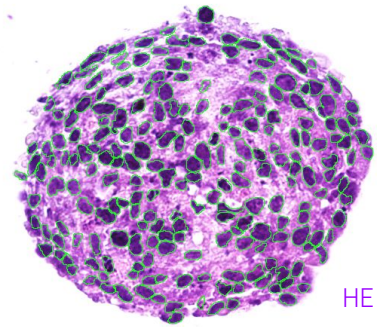
Organoid in 96-well plate → Organoid fixation → Embedding (FFPE block) → Automated staining (HE/IHC/IF/ISH/FISH) → High resolution scanning (40x) → 2D histology of in vitro model

Dry lab



AI algorithm for nuclei segmentation

VISIOPHARM® AUGMENTED PATHOLOGY



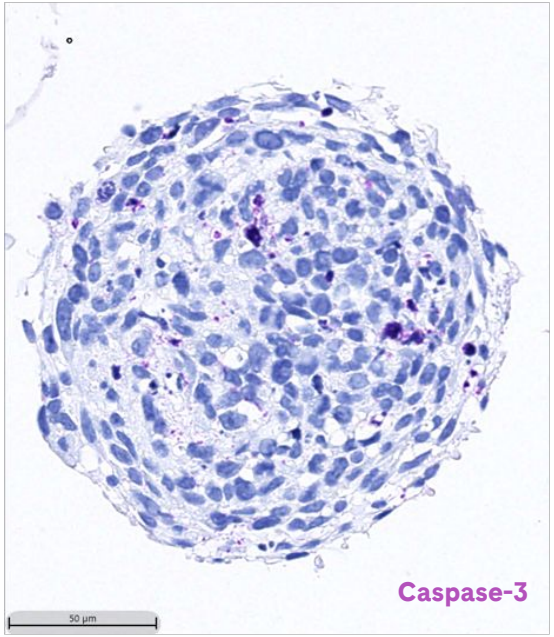
Quantitative and qualitative evaluation of nuclei segmentation

Bell et al., in preparation

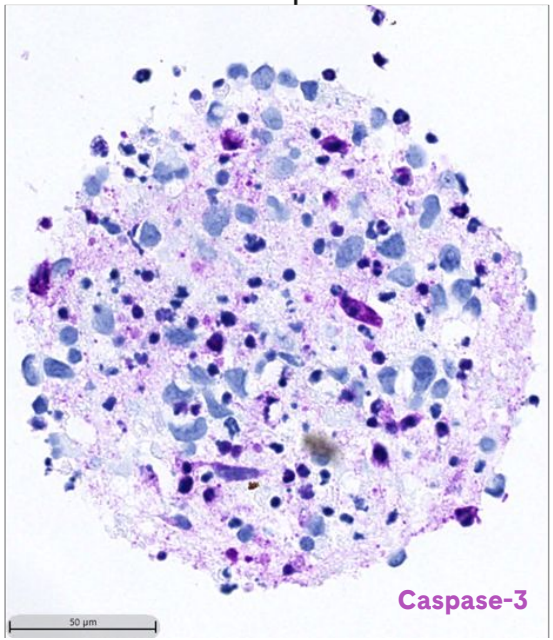
Pathology readouts of CIVMs

Get to know your model you work with - characterization for the context of use is crucial!

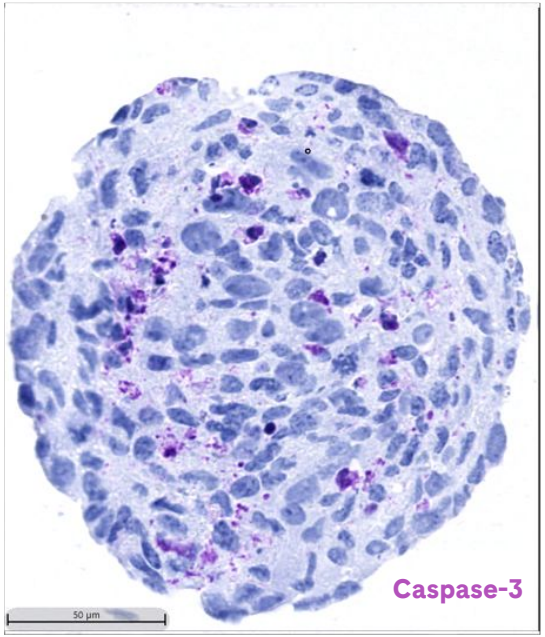
Wildtype BBB spheroids



Wildtype BBB spheroids + Staurosporine



Tumor-BBB spheroids



Bell et al., in preparation

Histology-guided engineering and characterization of complex *in vitro* model of blood-brain barrier for efficacy/toxicity in drug development

Luisa Bell[1,3], Martina Pigoni[2], Roberto Villaseñor[2], Jose Galvan[1], Claire Simonneau[1], Chiara Zanini[1], Christelle Zundel[1], Petra Stäuble[1], and Nadine Stokar-Regenscheit[1]

Roche Pharma Research and Early Development (pRED), [1]Pharmaceutical Sciences, [2]Neuroscience & Rare diseases, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland; [3]Pharmaceutical Sciences, University of Basel, Basel, Switzerland

Molecular pathology-guided characterization and validation

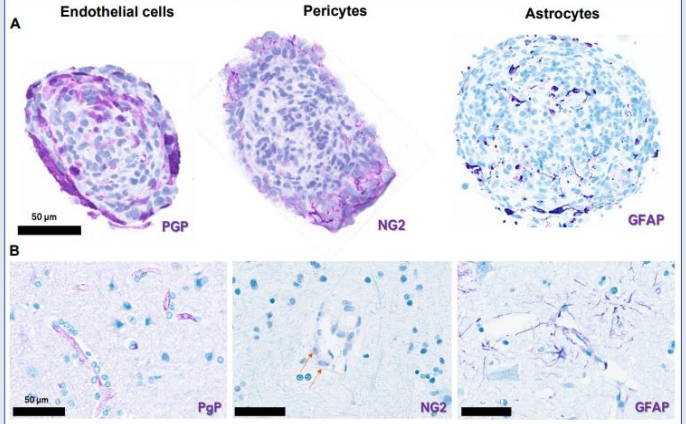


Figure 5. Molecular pathology-guided characterization of BBB organoids and validation of the markers towards human brain. A) Previously identified BBB markers for each cell type (PGP for brain endothelial cells, NG2 for Pericytes, GFAP for astrocytes) have been detected using IHC on BBB organoids and B) in the corresponding tissue of origin (human brain) for validation.

Complex organoid assembly and architecture

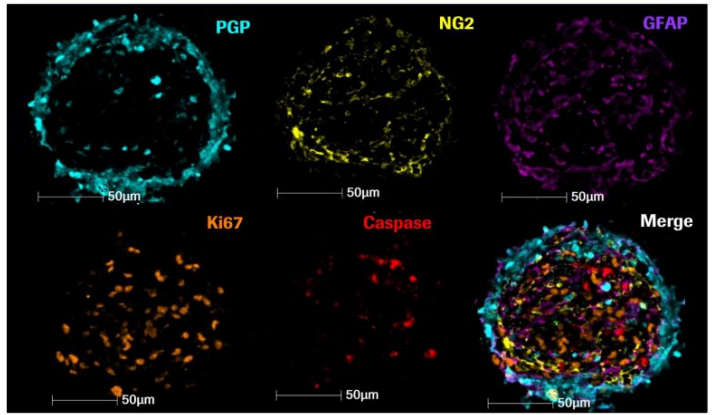


Figure 6. Model validation with multiplex immunofluorescence. By combining all relevant cellular makers (PGP for brain endothelial cells, NG2 for Pericytes, GFAP for astrocytes, Ki67 for proliferating cells and Caspase-3 for apoptotic cells) BBB organoids assembly and architecture can be assessed using automated sequential multiplex immunofluorescence assay (Ventana DISCOVERY ULTRA Autostainer, Opal dyes from Akoya Bioscience) followed by multispectral imaging with spectral unmixing (Vectra Polaris/Perkin Elmer). For establishment, optimal antibody order and corresponding opal dyes as well as denaturation/neutralization steps were tested and selected. Note the increased amount of proliferating and apoptotic cells which is usually not evident in a physiological brain, but a normal background in CIVMs.

Compound detection in organoids

As a proof of concept, we applied our newly developed histotechniques to detect compounds in organoids which then can be used for efficacy/toxicity studies during preclinical drug development. As a tool compound, we used a BrainShuttle, a monovalent antibody against the human transferrin receptor.

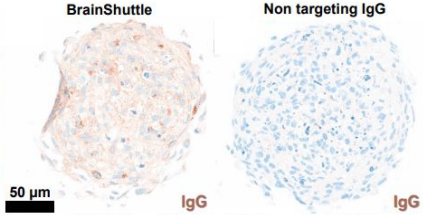


Figure 7. Human IgG IHC of BBB organoids detecting the BrainShuttle vs. non targeting IgG. BBB organoids have been treated with BBB targeting BrainShuttle or non-penetrating IgG for 4h, respectively. By IHC, we can identify the spatial distribution of BBB penetrating antibodies in BBB organoids.

Discussion and Conclusion

- The BBB model consists of three relevant cell types: **brain endothelial cells, pericytes and astrocytes**
- Cellular **spatial distribution** supports **functional characterization** of an intact BBB
- Compared to human tissue, the BBB organoid contains **apoptotic, proliferating and atypical cells**
- In future, histotechniques can be further **expanded to other CIVMs** and used for **efficacy and safety assessment** in preclinical drug development

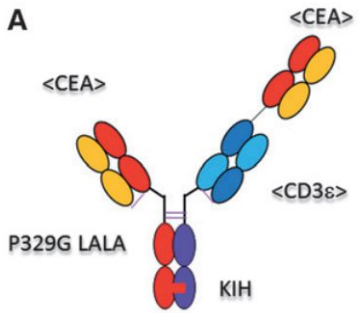
Contact

Luisa Bell, PhD Student
luisa.bell@roche.com

NAMs toolbox for combined safety / efficacy assessment of tumor targeting TCBs

Case example: Spatiotemporal readouts of PBMC co-cultured gut organoids

Carcinoembryonic Antigen (CEA) T-cell bispecific antibody (TCB)[25]



Cancer Therapy: Preclinical

Clinical Cancer Research

A Novel Carcinoembryonic Antigen T-Cell Bispecific Antibody (CEA TCB) for the Treatment of Solid Tumors

Marina Bacac[1], Tanja Fauti[1], Johannes Sam[1], Sara Colombetti[1], Tina Weinzierl[1], Djamila Ouaret[2], Walter Bodmer[2], Steffi Lehmann[3], Thomas Hofer[4], Ralf J. Hosse[4], Ekkehard Moessner[4], Oliver Ast[4], Peter Bruenker[4], Sandra Grau-Richards[4], Teilo Schaller[1], Annette Seidl[5], Christian Gerdes[1], Mario Perro[1], Valeria Nicolini[1], Nathalie Steinhoff[1], Sherri Dudal[6], Sebastian Neumann[7], Thomas von Hirschheydt[8], Christiane Jaeger[4], Jose Saro[9], Vaios Karanikas[9], Christian Klein[1], and Pablo Umaña[1]

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CLINICAL STUDIES

Application of a MABEL Approach for a T-Cell-Bispecific Monoclonal Antibody: CEA TCB

Dudal, Sherri*; Hinton, Heather†; Giusti, Anna M.†; Bacac, Marina†; Muller, Magali*; Fauti, Tanja†; Colombetti, Sara†; Heckel, Tobias*; Giroud, Nicolas*; Klein, Christian†; Umaña, Pablo†; Benincosa, Lisa*; Bachl, Juergen*; Singer, Thomas*; Bray-French, Katharine*

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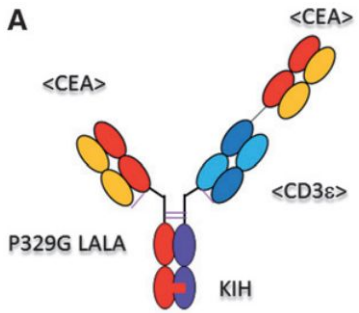
Carcinoembryonic Antigen (CEA) T-cell bispecific antibody (TCB)[25]



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Challenges in CEA-targeting TCB development

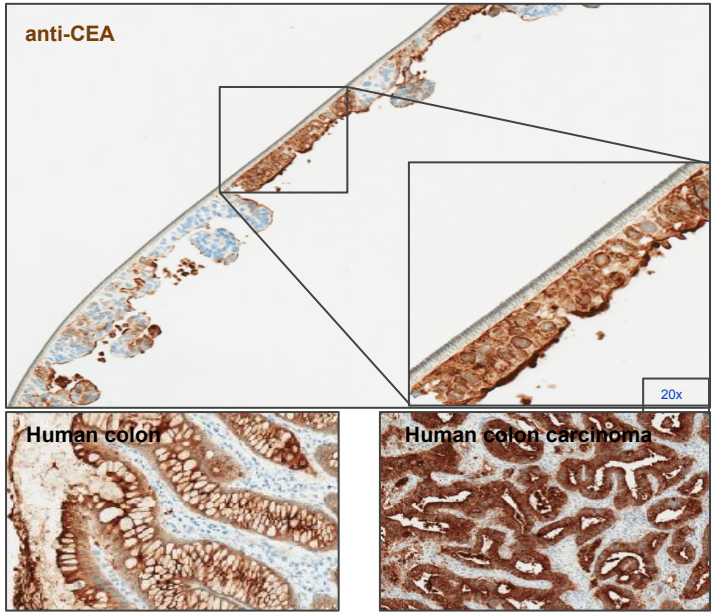
Clinical experience with Cibisatamab (CEA TCB): trigger (manageable) diarrhea in phase I clinical trials, suggesting on-target off-tumor intestinal reactivity, consistent with CEA expression in the healthy intestine

→ *need for predictive non-clinical models for future CEA-targeting TCBs*

Cibisatamab (RG-7802) is under development for the treatment of advanced metastatic CEA-positive solid tumors including metastatic colorectal cancer, squamous non-small cell lung cancer (first and third line therapy), breast cancer, pancreatic cancer, gastric cancer including gastroesophageal junction cancer. This antibody acts as a bi-specific T-cell engager. The drug candidate acts by targeting carcinoembryonic antigen (CEA, CEACAM5, and CD66e) antigen on tumor cells and CD3 on T cells. [26]

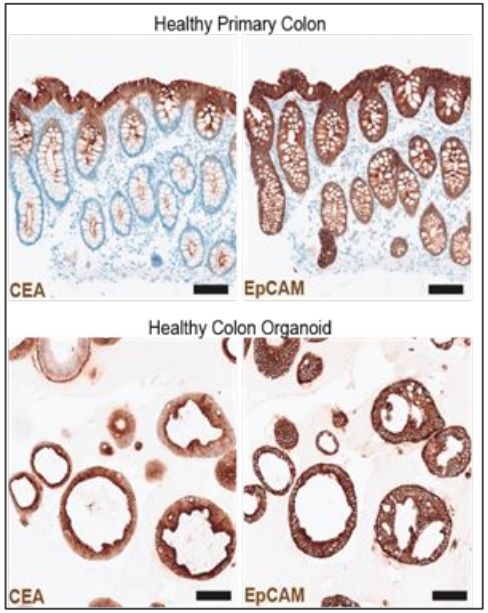
In vitro models & readouts for CEA-targeting TCBs

MatTek Intestinal epithelium (transwell)
(initiated from human gut biopsies)



Anneliese Schneider, Martin Lechmann, Heather Hinton, Cristina Bertinetti-Lapatki, Anna Maria Giusti

Intestinal epithelium organoids
(initiated from human gut biopsies)



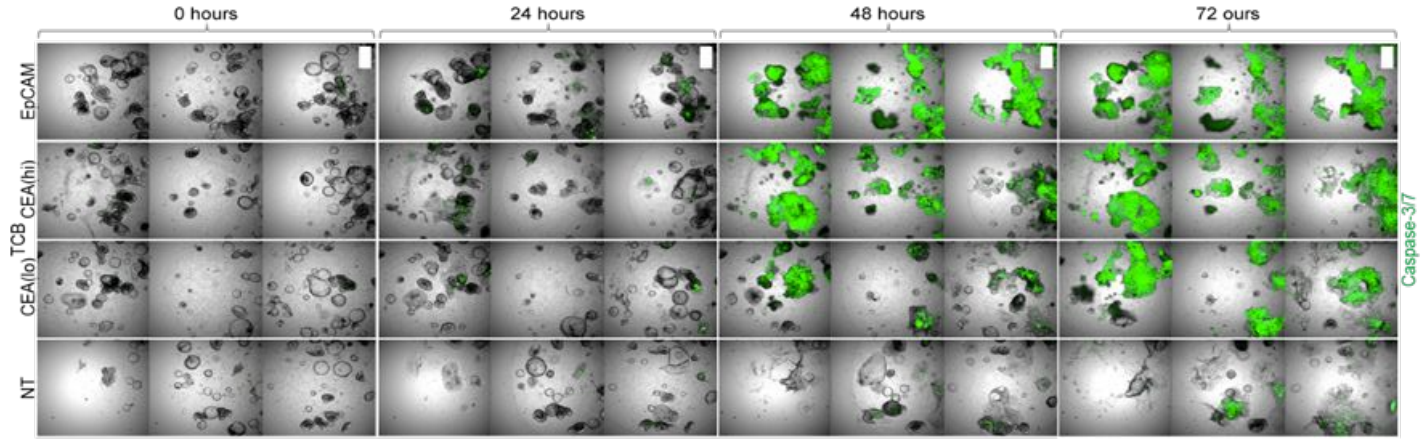
Harter et al., 2023 [27]

Why were on-target GI effects not reflected in vitro?

Current limitations of organoid models in preclinical safety pipeline

- Organotypic properties of organoids often not validated
- Organoids often lack indispensable immune compartment for capturing TCB-mediated effects
- No adequate assays / readouts to capture multifactorial TCB-triggered toxicity
- Most assays focus on epithelium, without mechanistic insight of interaction between epithelial and immune cells
- Usually low-throughput assays without critical spatial dissection

Standard 5x Caspase (killing) readout (Ex: gut organoids)



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2020, VOL. 17, NO. 1, 67–85
https://doi.org/10.1080/1547691X.2020.1729902

Taylor & Francis
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RESEARCH ARTICLE

OPEN ACCESS Check for updates

Summary of a workshop on preclinical and translational safety assessment of CD3 bispecifics

Cris Kamperschroer[a*], Jacintha Shenton[b*], Hervé Lebrec[c], John K. Leighton[d], Paul A. Moore[e] and Oliver Thomas[f]

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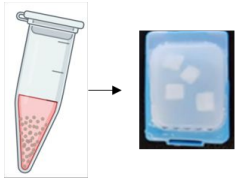
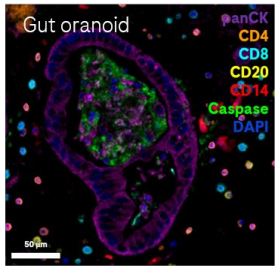
Pathology readouts of PBMC co-cultured gut organoids

Molecular pathology guided model engineering, characterization and morphologic toxicity readouts of a novel PBMC co-cultured gut organoids for the purpose to assess on-target toxicity

Molecular pathology guided engineering

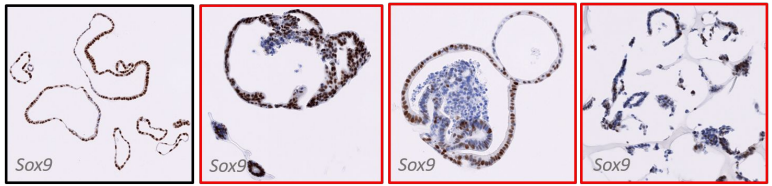


Fixation & Embedding (FFPE)

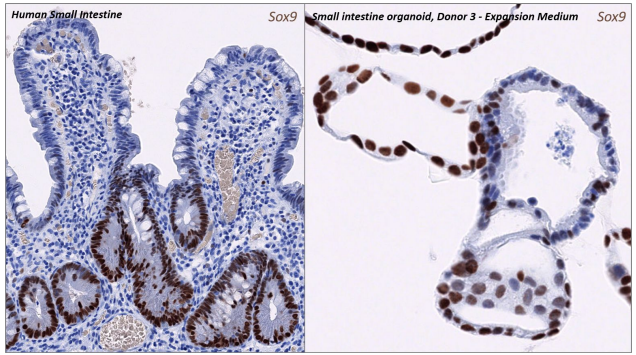


Guide optimization / engineering of the model

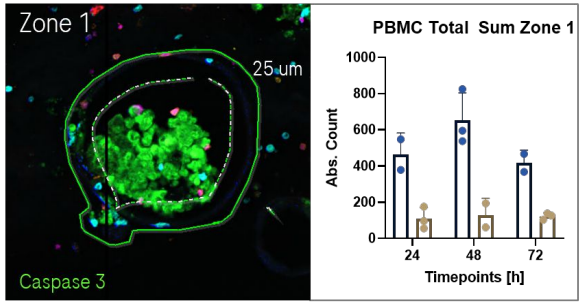
Characterization of human gut organoid differentiation over time to assess the best treatment time point



Characterization & validation towards human tissue



Automated high resolution toxicity readouts

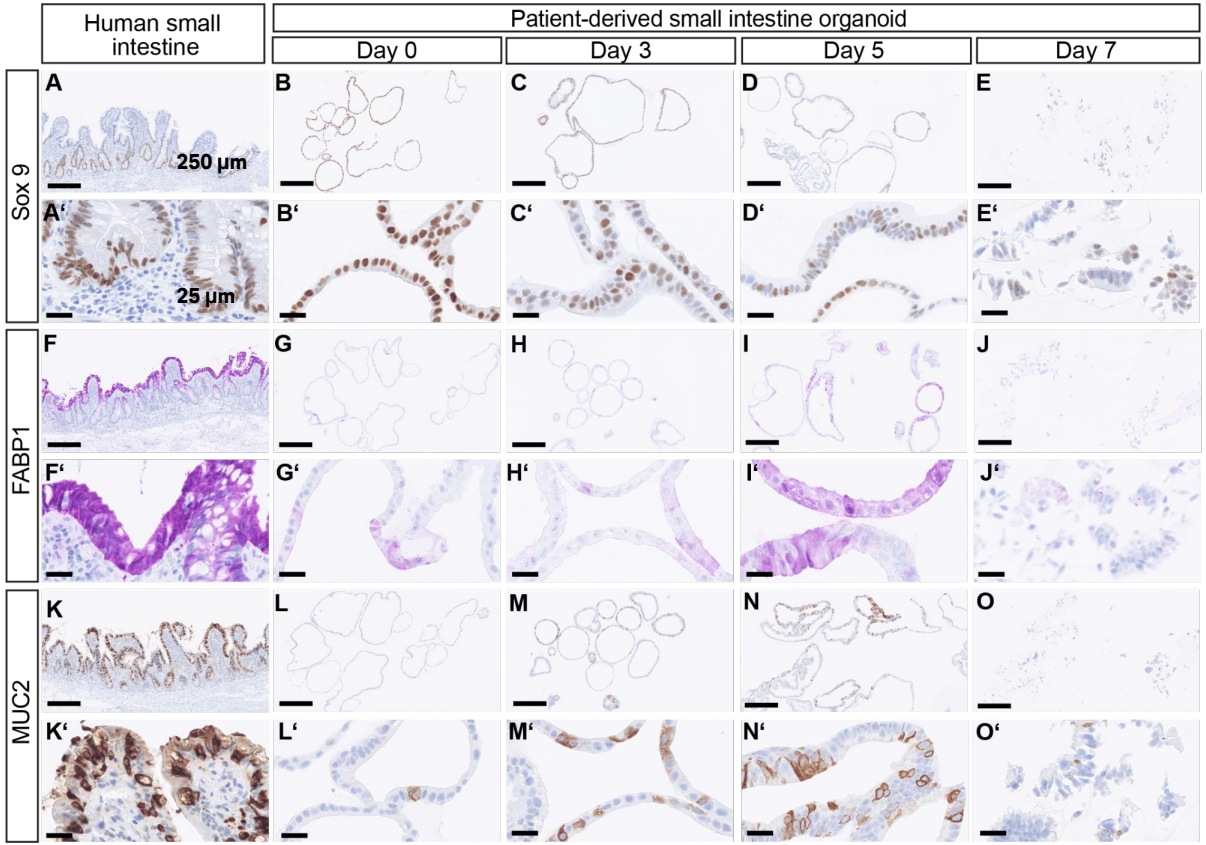


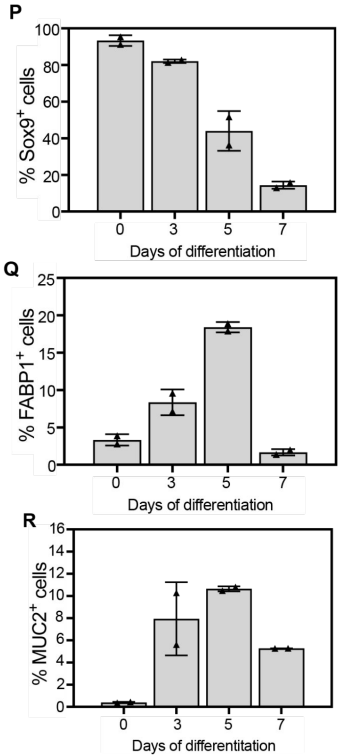
Spatial quantification of PBMCs / e.g. CD8⁺ T cells in CEACAM5 TCB treated gut organoids

Harter et al, 2023 [27]

Model characterization & evaluation of the best treatment timepoint

Gut organoids - characterization towards human small intestine tissue by Immunohistochemistry (IHC)

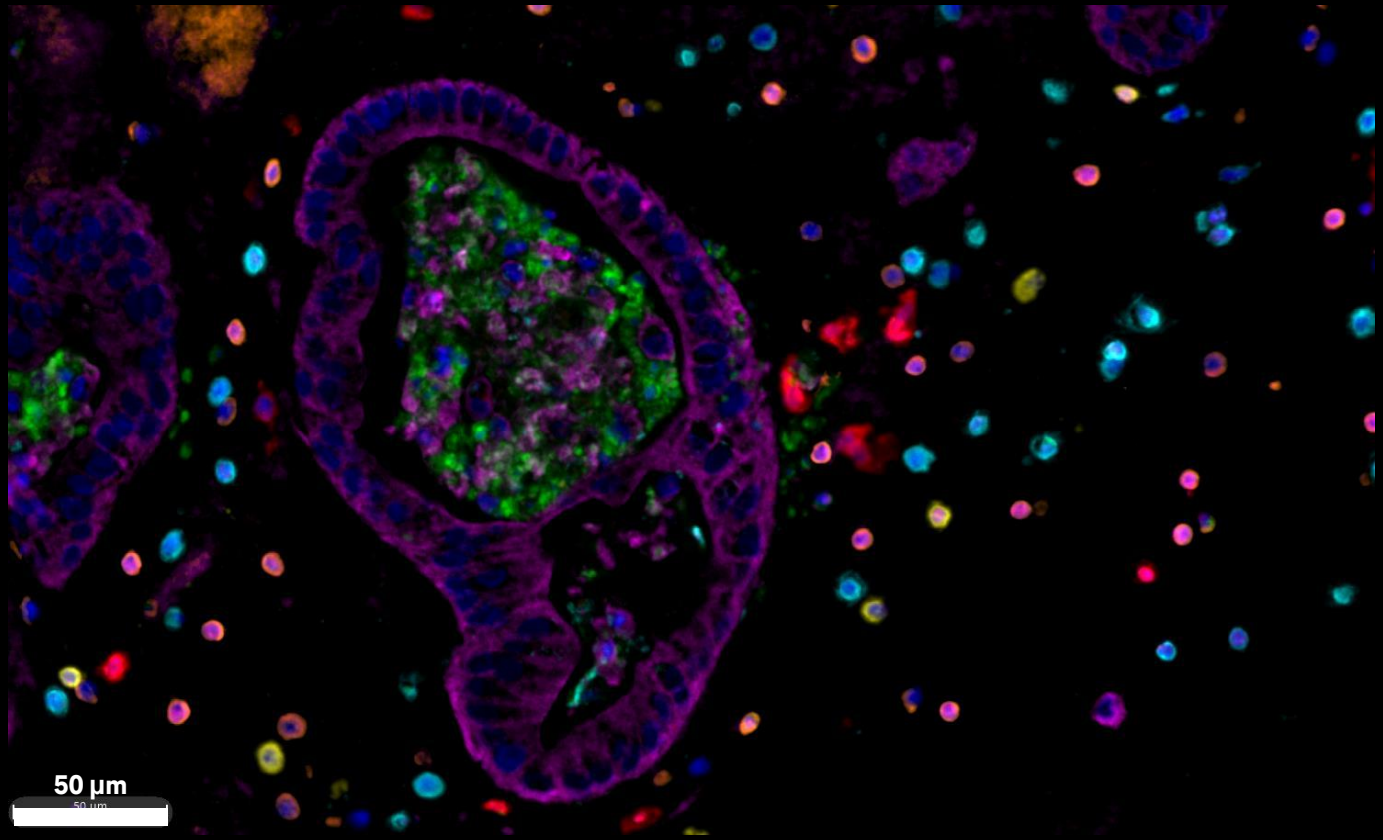




Harter et al, 2023 [27]

Model characterization with multiplex immunofluorescence

Retention of spatial location of PBMCs co-cultured intestinal organoids

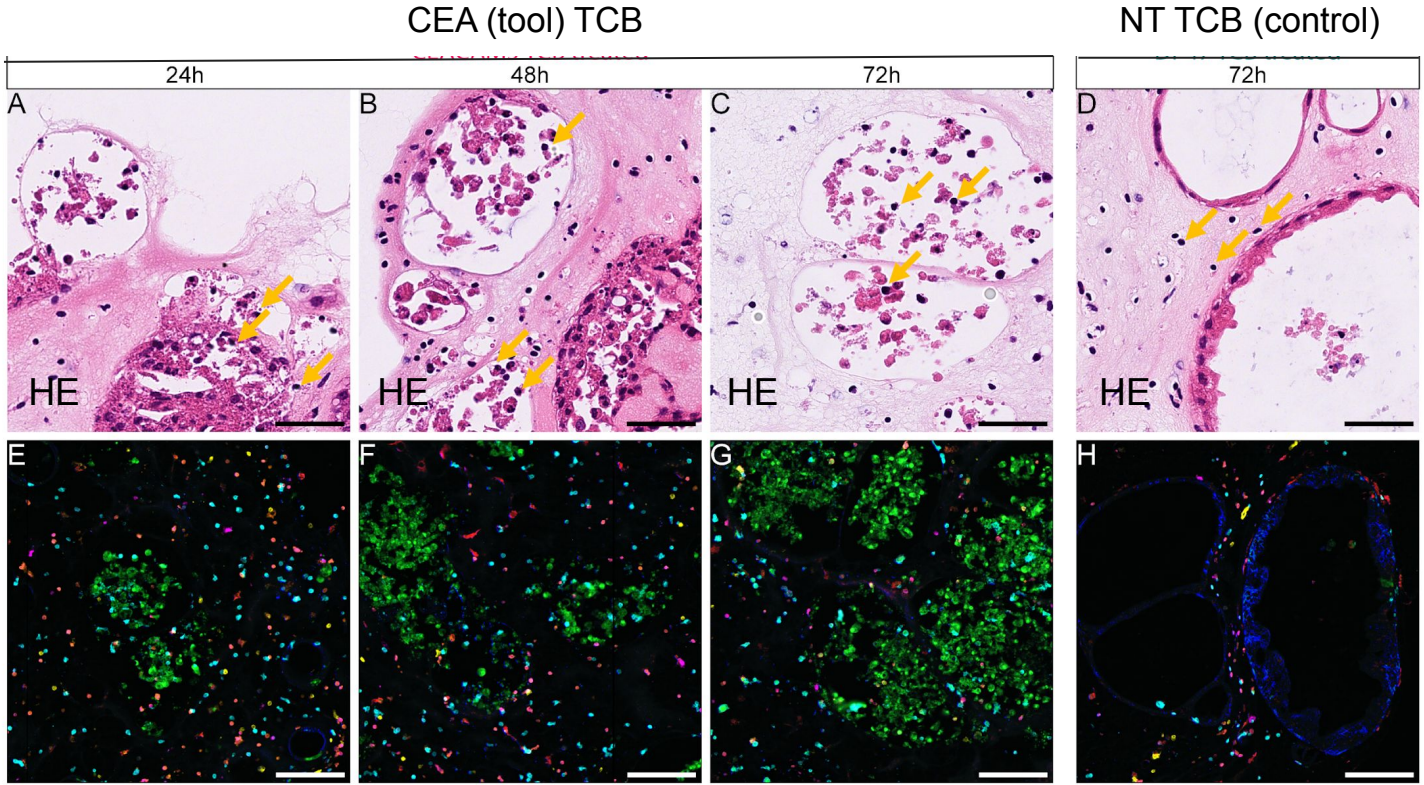


panCK
CD4
CD8
CD20
CD14
Caspase
DAPI

Harter et al., 2023

CEA (tool) TCB mediated killing in PBMC co-cultured gut organoids

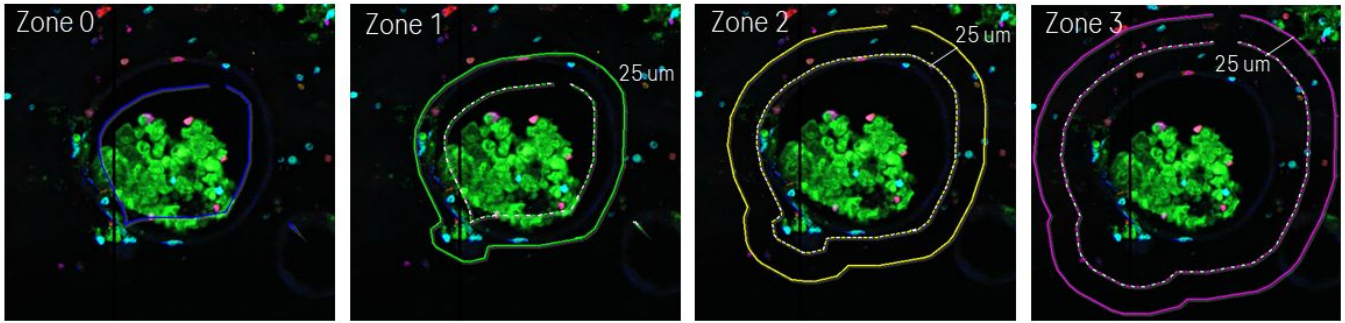
Histology (HE) and Multiplex Immunofluorescence (IF) readouts

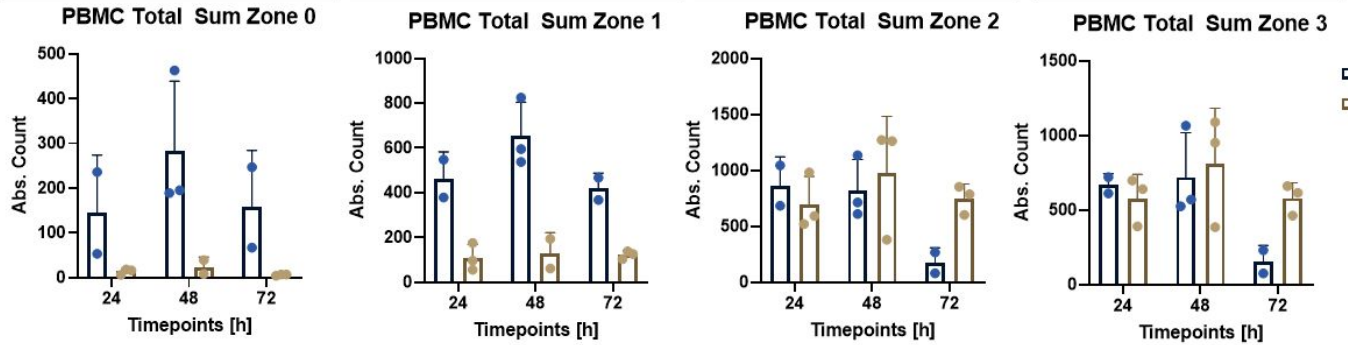


Harter et al, 2023 [27]

CEA (tool) TCB mediated killing in PBMC co-cultured gut organoids

Spatiotemporal evaluation of PBMCs - gut epithelium interaction





Harter et al, 2023 [27]

Summary & Conclusion

Results

- PBMCs co-cultured colon organoids & spatial readouts allow off-tumor on-target toxicity evaluation in vitro
- Pathology expertise and tissue technologies / AI-based morphological image analysis as key readouts for CIVM engineering, model characterization and toxicity evaluation

Implications

- Fit for purpose model characterization is key (need to understand the limitations!)
- Readouts and non-clinical models need to be co-created
- More than one tool (NAMs, e.g. GEMM, CIVM, in silico etc.) may be needed for predictive translation to human
- Regulatory frame to be set

Opportunities

- Leveraging new technologies allows innovative model development (GEMMs & CIVMs) & characterization
- Combine efficacy & safety readout in the same non-clinical models
- Back-translation efforts allow improvement of models and readouts
- Scale approach for other targets & organ systems

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(PhD Thesis Luisa Bell)

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Regulatory Guidance documents associated with NAMs

Regulatory Guidances

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ICH S5(R3) Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals Guidance for Industry, 2021 – Description of testing strategies utilizing alternative assays for the assessment of malformations and embryofetal lethality, and the qualification process for these alternative assays.

ICH M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals To Limit Potential Carcinogenic Risk Guidance for Industry, 2018 – Use of computational approaches for the assessment of mutagenic potential of drug impurities.

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