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Daiichi-Sankyo

Utilization of Human Based Microphysiological Systems and Complex In Vitro Models in Drug Discovery and Development: Perspectives from Pharmaceutical Industry

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COI disclosure:

Veronika Rozehnal is an employee of Daiichi Sankyo

Outline

- Definition of CIVM and MPS
- Driving Forces for Alternatives to Animal Testing
- Daiichi Sankyo’s Tissue and Cell Research Center Munich (TCRM)
- Human models in current and past application at TCRM
  - Human Tissue-derived Intestinal Organoids
  - Human Kidney Model
  - Viable Human Tissue Slices
- Perspectives of application of MPS/CIVM in pharmaceutical industry
- Conclusion

What are Complex In Vitro Models (CIVM) and Microphysiological Systems (MPS)

Microphysiological systems (MPS) comprise a number of bioengineering breakthroughs that reproduce organ architecture and function *in vitro*. Fueled by stem-cell technologies, a broad variety of especially human models and test systems have emerged, which make relevant experimental tools broadly available through international and multidisciplinary collaborations. *(Definition by International MPS Society)*

A broad definition of MPS includes the **Complex In Vitro Models (CIVM)**. CIVM can be defined as either spheroids, organoids, three-dimensional bioprinted tissue, organs-on-a-chip or MPS, or a multi-organ system or human body on a chip. *(Baran et al, 2022, Ekert et al. 2020)*

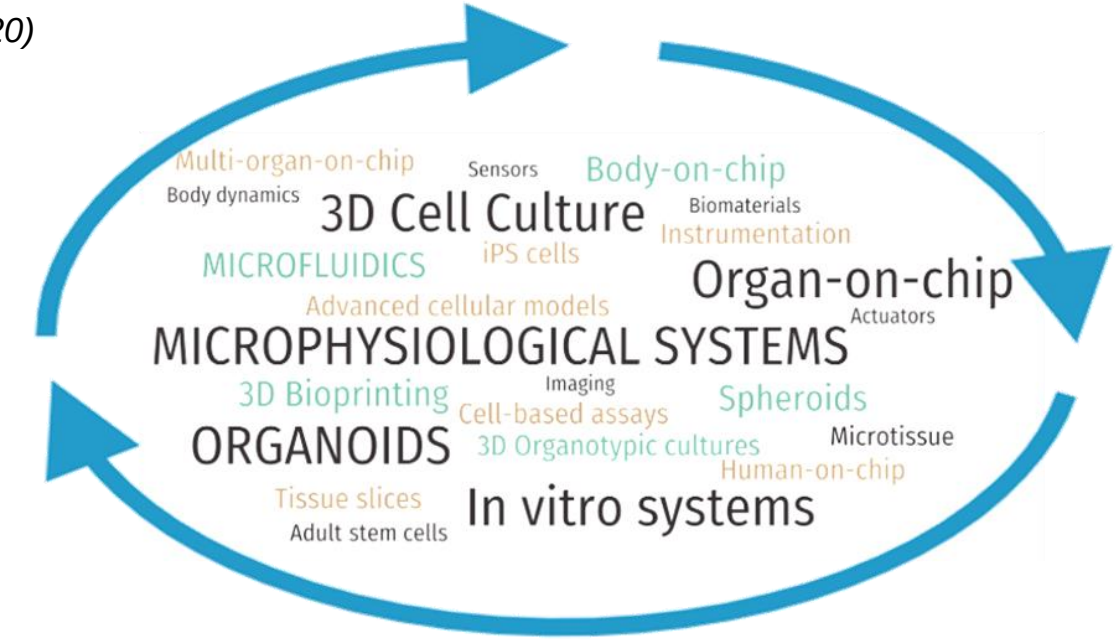


Figure by International MPS Society, modified

Driving Forces Supporting Alternatives to Animal Testing

The driving forces behind a shift towards alternatives to animal testing, including MPS/CIVM technologies, are **human relevance**, **cost reduction**, **ethical considerations** and **laws and regulations**.

FDA Modernization Act 2.0

- Eliminates the requirement of animal testing before initiation of clinical trials
- Provides opportunities to use alternatives to animal testing in drug discovery and development
- Those alternatives are in silico methods, in vitro methods, and MPS/CIVM technologies
- The Act does not eliminate animal testing and FDA's ability to require it when necessary

European Commission's Roadmap for Phasing-out Animal Testing

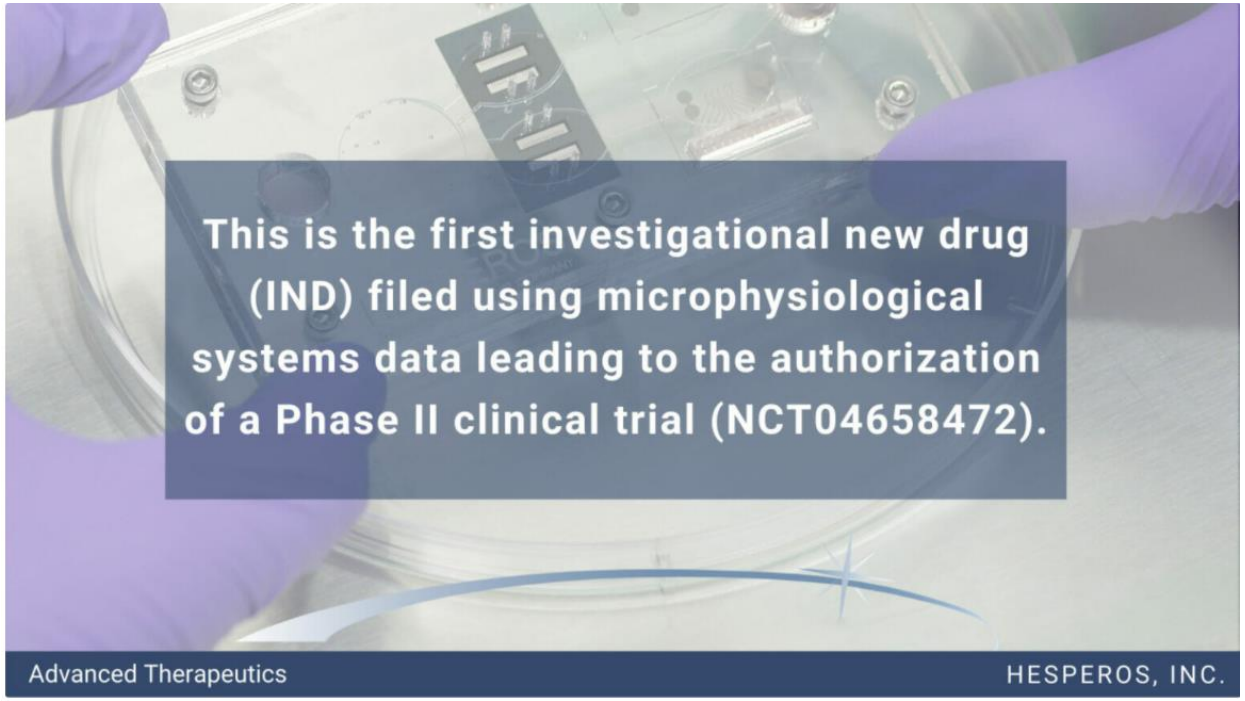
- Acknowledges animal welfare as an important concern of European citizens
- Proposes actions to further reduce animal testing (in addition to full ban on cosmetic testing in 2013)
- New roadmap with legislative and non-legislative actions towards an animal-free regulatory system
- Emphasis on modernization of science and strong support for research in developing alternatives

https://www.congress.gov/bill/117th-congress/senate-bill/5002
https://ec.europa.eu/commission/presscorner/detail/en/ip_23_3993

Break through in regulatory acceptance of MPS

HESPEROS
THE HUMAN-ON-A-CHIP COMPANY

SANOFI



- Hesperos showed potential to replace conventional animal efficacy studies for rare neuromuscular disorders
- Validated use of MPS system to mimic disease mechanisms of rare autoimmune neuropathies not replicable in animals
- Efficacy data from MPS system supported the authorization of a clinical study in 2021 through collaboration with Sanofi

Press release Hesperos 2022

Tissue and Cell Research Center Munich (TCRM)

The non-clinical research site of Daiichi Sankyo in Europe

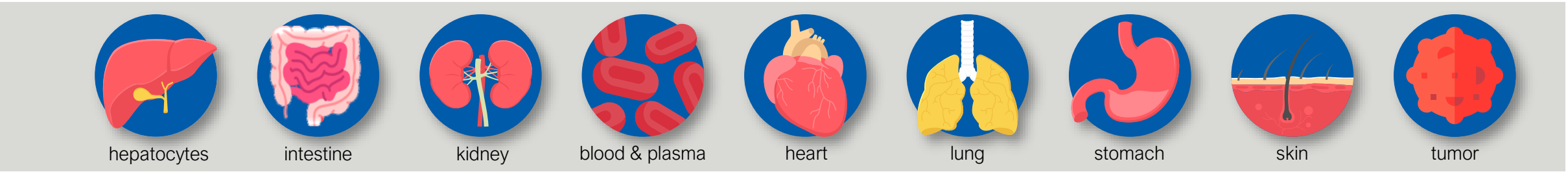
- Established in Germany in 1998, part of Daiichi Sankyo's global R&D
- Focusing on translational research using human biospecimens
- Works exclusively with human models as alternatives to animal testing
- Broad network of academic and commercial human tissue providers
- Initial focus: drug metabolism, drug absorption, drug-drug interaction
- Expanded focus: MoA, mechanistic investigation, biomarker research

Mission: Enhance the prediction accuracy of drug candidates' efficacy and safety by advancing the utilization of human models bridging the gap between experimental animals and clinical trials.

This presentation focuses on models applied at TCRM not Daiichi Sankyo Co. Ltd.

MoA = Mechanism of Action

Acquisition of viable human tissue at TCRM

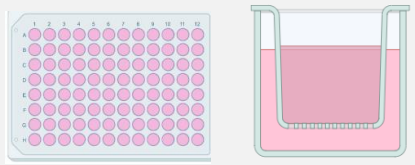


- Samples obtained from network of providers are from discarded surgical tissues
- Patients undergoing partial excision of organ, mostly due to cancer
- Written donor consent is given, no profit for patients, pseudonymised samples
- Donor information (e.g. age, gender, BMI, diagnosis, medication) available
- All samples tested negative for HIV, hepatitis B virus, hepatitis C virus
- Fresh tissues transferred to TCRM´s laboratories immediately after surgery
- Collection of tissues approved by the local responsible Ethics Committee
- Research on basis of World Medical Association Declaration of Helsinki

Human models in current and past application at TCRM

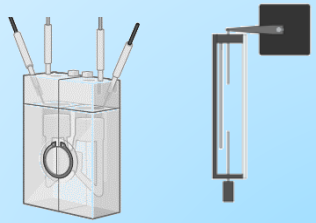
Conventional assays ⟵ ⟶ *Advanced human models, CIVM/MPS*

2D cell culture*



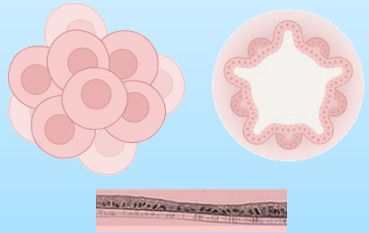
- ✓ *Hepatocyte culture*
- ✓ *Renal cells culture*
- ✓ *Others*

Tissue Incubations*



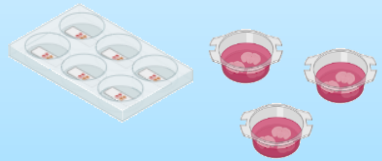
- ✓ *Ussing Chamber*
- ✓ *Tissue Bath*
- ✓ *Functional assays*

Organoids, Spheroids



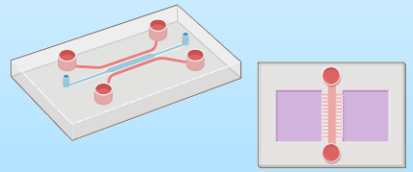
- ✓ *Intestinal organoids*
- ✓ *Tumor organoids*
- ✓ *Tumor spheroids*

Viable Human Tissue Slices



- ✓ *Tumor, non-tumor*
- ✓ *Static culture*

MPS devices



- ✓ *Cells and organoids*
- ✓ *Viable tissue slices*
- ✓ *Tumor, non-tumor*

<u>Criteria for utilizing advanced human models:</u>

- Clear advantage over conventional models
- Species differences or lack of suitable animal models (e.g. human target specific drugs)

<u>Examples of utilizing advanced human models:</u>

- Mechanistic investigation in pharmacology / toxicity
- Evaluation of immunological mechanisms
- Disease models and Biomarker research

* Out of scope of today's presentation; Graphic created with BioRender.com

Human Tissue-derived Intestinal Organoids

Preparation: *(Stemcell® Protocol)*

- TCRM uses human small intestine and colon tissue for isolation of crypts

Application:

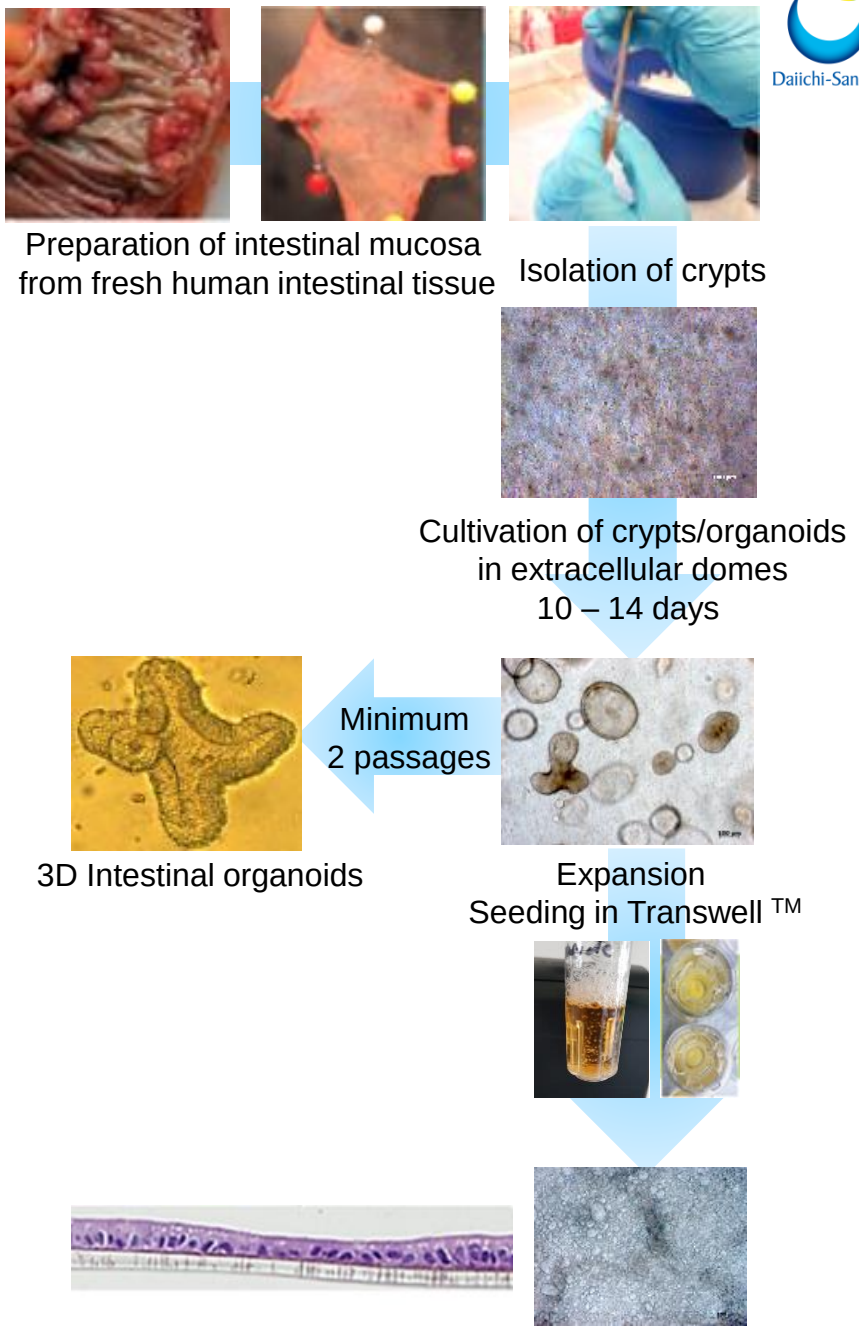
- Prediction of gut bioavailability (F_aF_g)
- Induction of intestinal CYP3A4 and P-gp (*ABCB1*)
- Assessment of intestinal safety of drugs

Advantages:

- Key intestinal features retained (crypts, villi, enzymatic activity)
- Inducibility of enzymes and drug transporters (CYP3A4 and P-gp)
- 3D configuration and 2D configuration in monolayer available
- Comparison between different intestinal segments possible

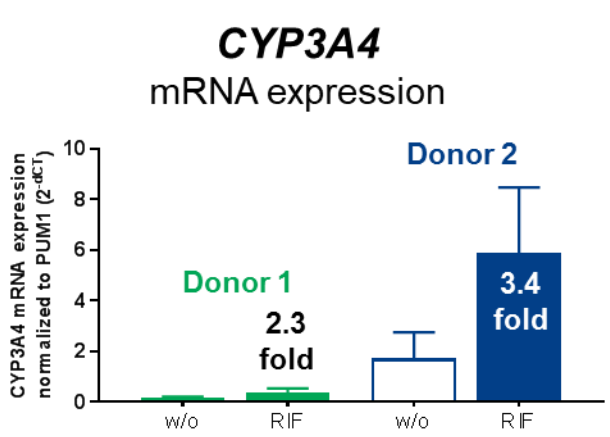
Limitations:

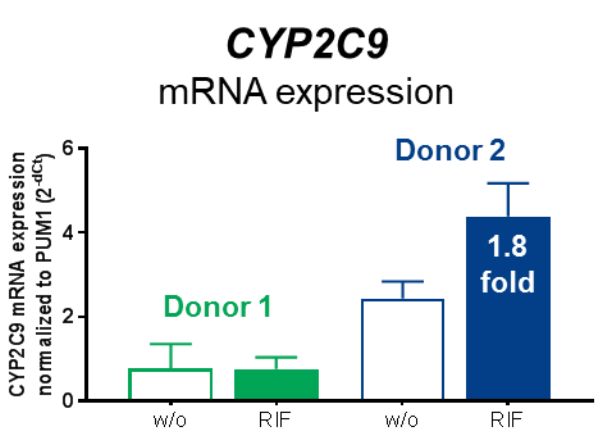
- Need further investigation on reproducibility and inter-donor variability
- Absence of vasculature and mechanical stimuli present *in vivo*
- Elevated costs of maintaining organoids in culture

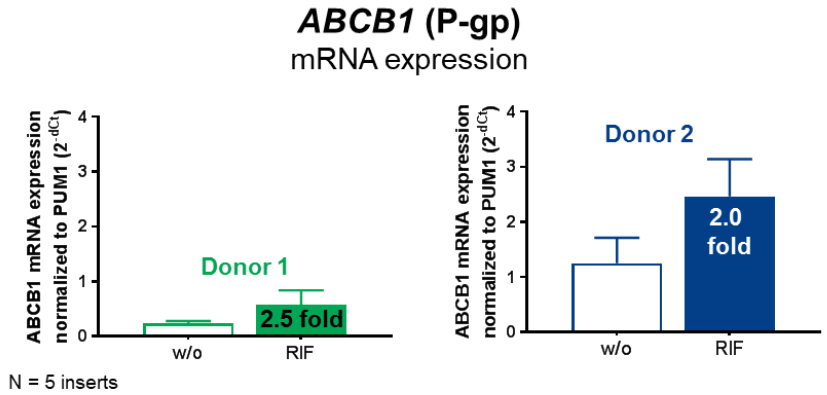


Sato et al. 2011, Zietek et al. 2015, Joseph et al. 2018, Michiba et al. 2022; Photos in house, Graphic created with BioRender.com

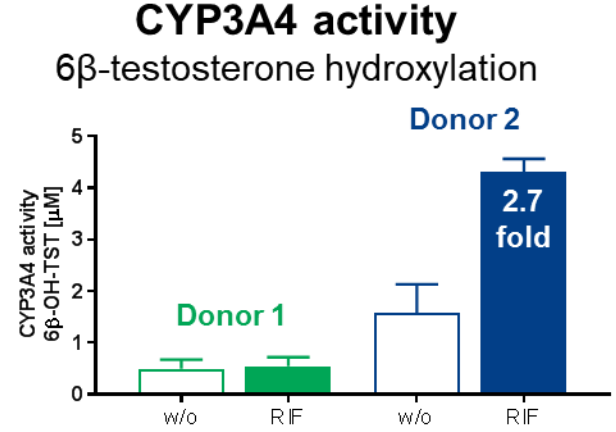
Human Tissue-derived Small Intestinal Organoids for ADME

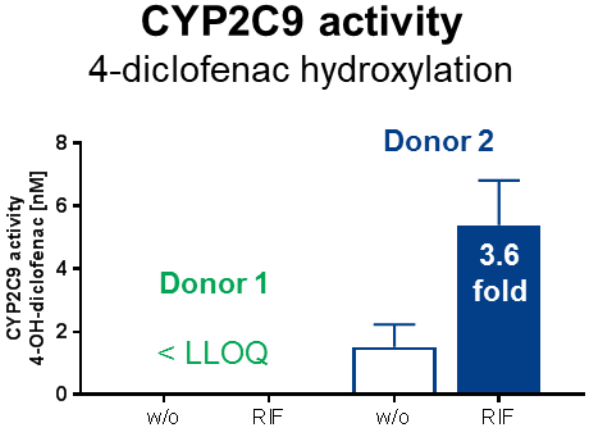


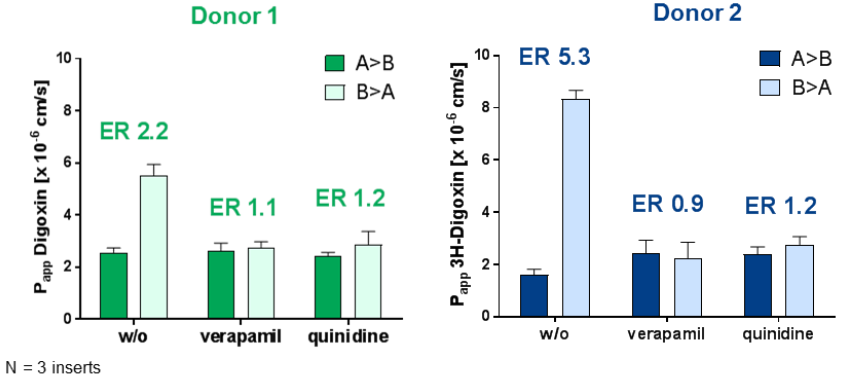




N = 5 inserts







N = 3 inserts

- ✓ mRNA expression of CYP450 metabolic enyzmes and drug transporters shown
- ✓ Metabolic activity of CYP450 enzymes inducible after treatment with rifampicin (RIF, 30 µM, 48 h)
- ✓ P-gp mediated transport of digoxin and it‘s inhibition by verapamil (50 µM) and quinidine (30 µM) shown

LLOQ: Lower Limit Of Quantification, CES: Carboxylesterases, RIF: Rifampicin P_{app} : Apparent Permeability ER: Efflux Ratio (BA/AB)

Human Kidney Model (Static and MPS)

Used cells:
- Freshly isolated renal proximal tubule cells (RPTEC) in-house
- Commercially available cryopreserved primary or immortalized RPTEC

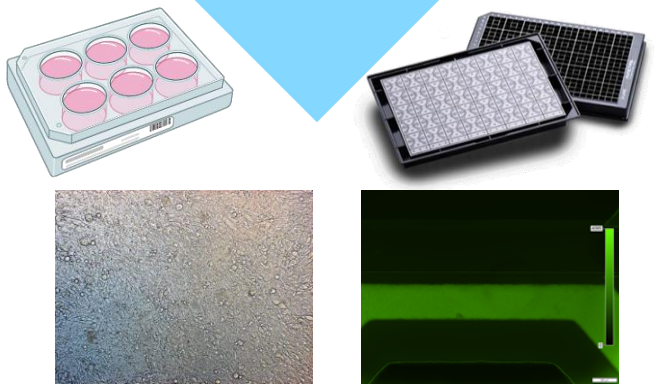
Goal:
- Prediction of human renal clearance and renal DDI
- Evaluate renal secretion and re-absorption simultaneously
- Assess inter-species differences in renal clearance

General observations:
- All types of RPTEC loosed transporter expression over time
- RPTEC in static culture might be suitable to evaluate OCT2 vs. MATE-driven DDI
- Cryopreserved and immortalized RPTEC show low initial transporter expression
- Freshly isolated RPTEC displayed different growth and attachment between donors

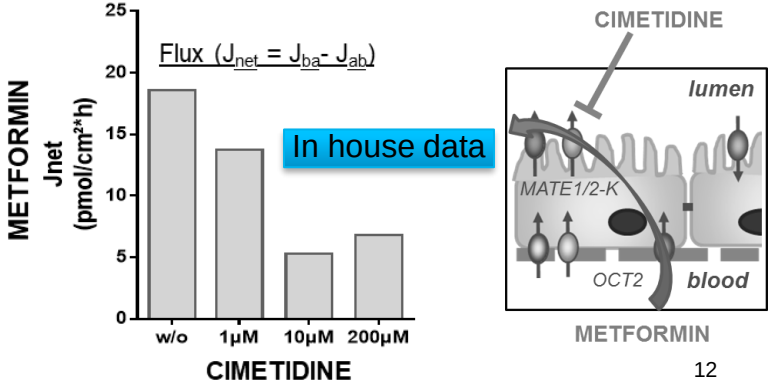
In the MPS device:
- Immortalized human RPTEC showed no organic anion/cation secretion
- Growing freshly isolated RPTEC in MPS device was very challenging

Conclusion:
- No functional differences between cells seeded in static model or MPS
- Transporter expression is decreased or lost in all cultures
- Kidney model discontinued

Fresh renal cortex minced and digested with collagenase.
RPTEC separated by density centrifugation



Seeding in Transwell ™ Seeding in Organoplate®

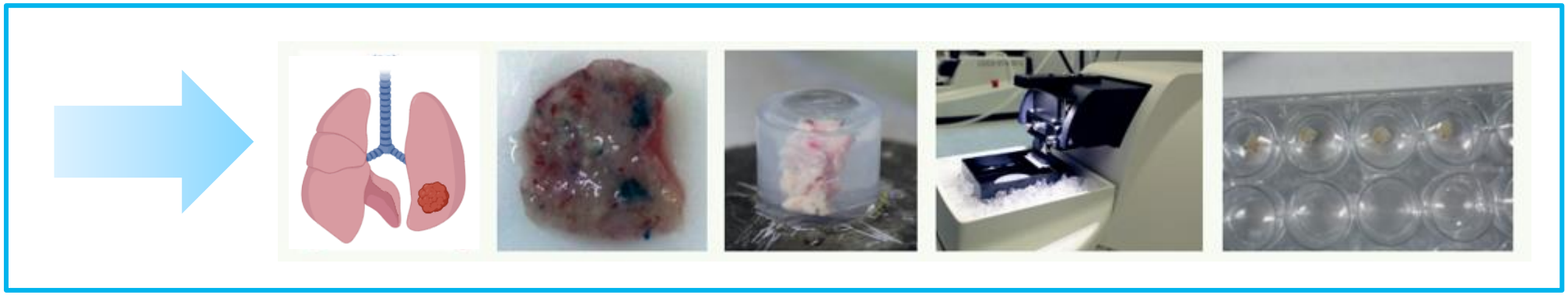


Brown et al. 2008, Van Ness et al., 2017; Photos in house, BioRender, Mimetas Organoplate®

Viable Human Tissue Slices (tumor and non-tumor tissue)

Concept: *Ex vivo* model for translational research and mechanistic evaluations in pharmacology and safety

Advancing TCRM`s capability to obtain fresh human tissue and perform functional studies



Workflow: Fresh tissue collection from the hospital, agarose embedding, cutting by tissue slicer, cultivation

Advantages: Full spectrum of cell types with preserved cell interaction and function, histologically readable results

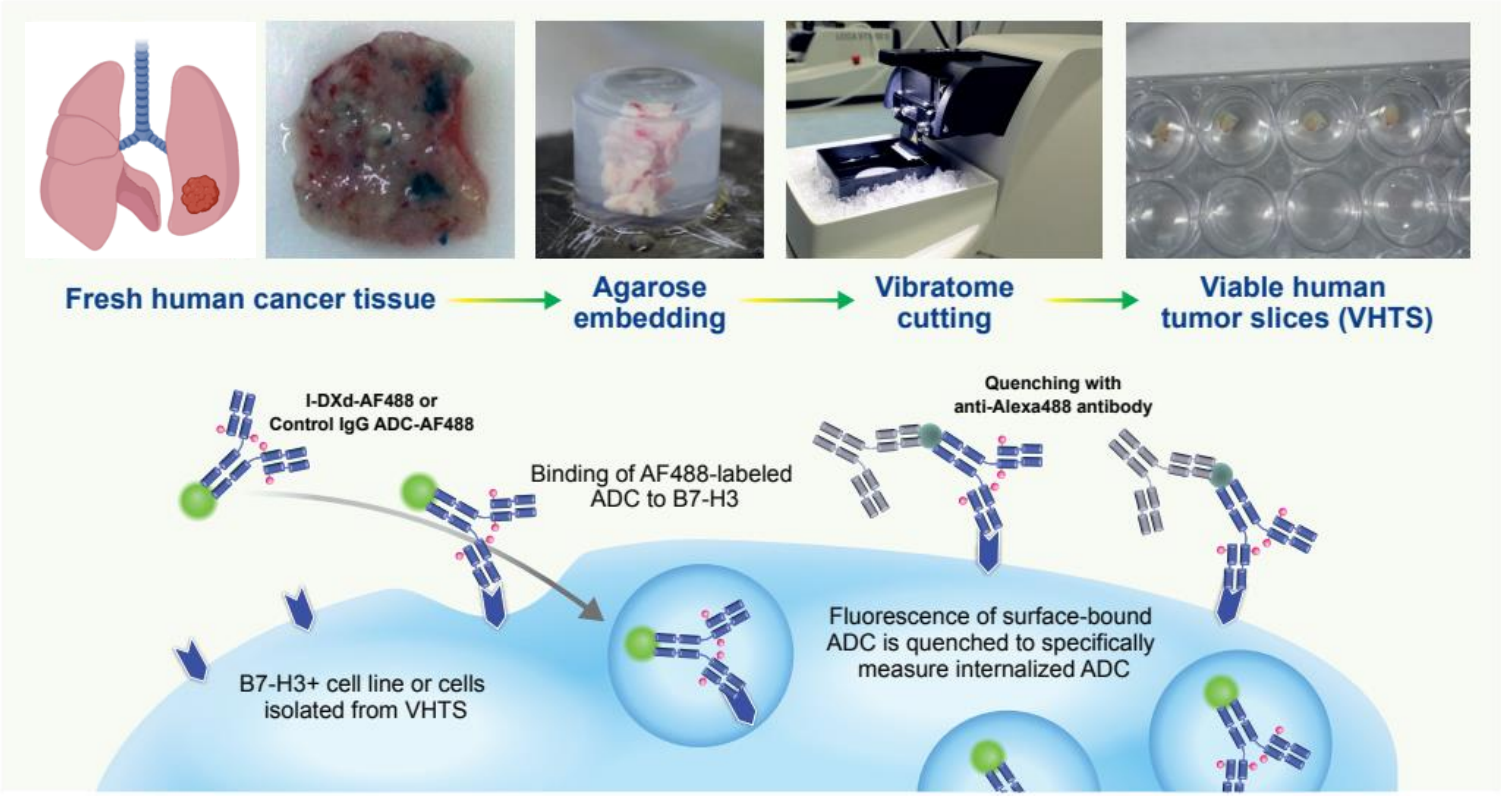
Read-outs: FCM, RNA sequencing, multiplex chemokine/cytokine analysis, histology, immunostaining, cytotoxicity

Objective: Effect on (non-)tumor cells, cell-type specific internalization, immune cell modulation, payload release, biomarker identification, target expression in (non-)tumor tissue, experiments with combination drugs

Van de Kerkhof et al. 2007, De Graaf et al. 2010; Photos in-house, Graphic created with BioRender.com

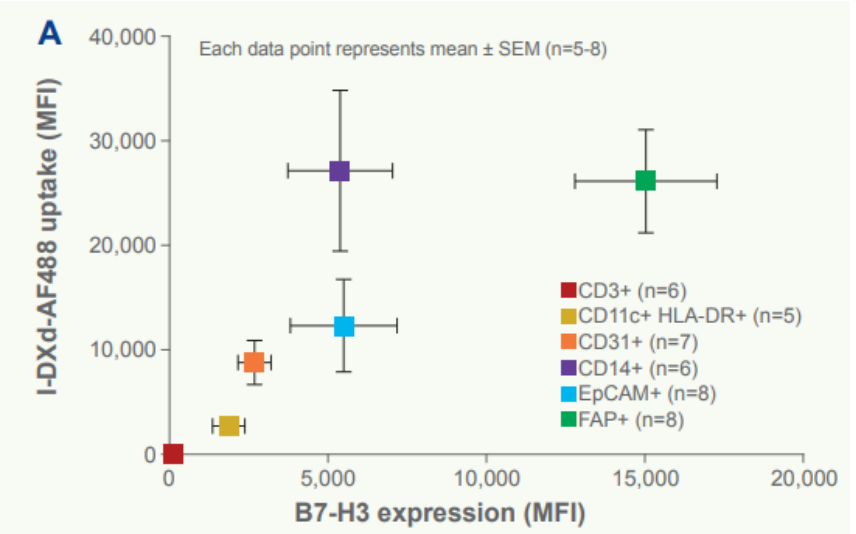
Viable human tumor slices for oncology research

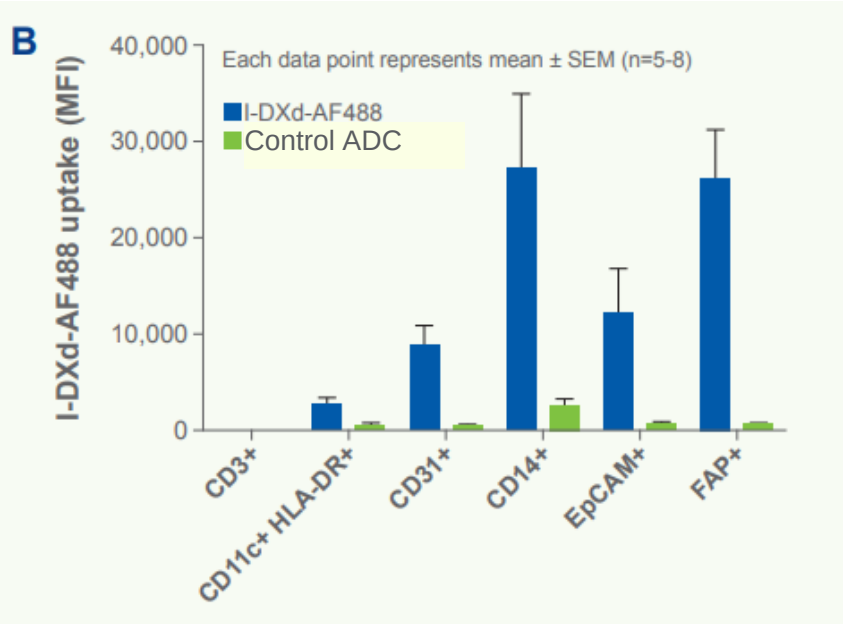
Target-dependent internalization of Ifinatamab deruxtecan (I-DXd)



Tumor slices prepared from fresh human non-small cell lung cancer tissue from eight patients and incubated with fluorescently labeled I-DXd or Control ADC, afterwards enzymatically digested and analyzed by flow cytometry.

I-DXd internalization was shown into B7-H3-expressing tumor cells and other cell populations in the tumor microenvironment demonstrated efficient payload delivery in a target-dependent manner.





Katsumata et al., AACR Annual Meeting, April 2023 (Poster)

Optimizing Viable Human Tumor Slices model : Tumor-on-a-Chip

Limitations of the static model

- X Restricted viability
- X Limited longevity
- X Insufficient complexity
- X Co-cultures challenging

Improvement in MPS

- Tumor and it’s microenvironment (TME) in dynamic culture
- Prolongation of the survival of tissue slices culture
- Co-culture approach more feasible
- Evaluation of drug effect on TME and immune cells

Photo in-house, Graphic created with BioRender.com

Perspectives of application of MPS/CIVM in pharmaceutical industry

Target selection and validation	Lead Identification	Lead Optimization	Preclinical safety	Clinical assessment
Target selection/validation in disease relevant models	Use where simpler models fail		Characterize adverse effects	Improve clinical trial design
Biomarker identification Mechanistic understanding	Disease modeling Disease-associated risk evaluation		Improve in vitro study design	Follow up human clinical findings
Identification of target organs	Complement or reduce animal studies		Species specific drug responses Help species selection	Predict patients specific drug response
Assessment of on/off target toxicity	Double-organ efficacy		Organ models for mechanistic safety	Organ models for reverse translational studies

Low et al., 2020; Kopec et al., 2021 modified

Conclusion CIVM/MPS

Opportunities:

- ✓ Are more predictive and physiologically relevant compared to traditional methods
- ✓ Can complement traditional models and make biomedical research more efficient and effective
- ✓ Are responsive to 3Rs principle of reducing, refining, and replacing the use of animals in nonclinical research

Challenges:

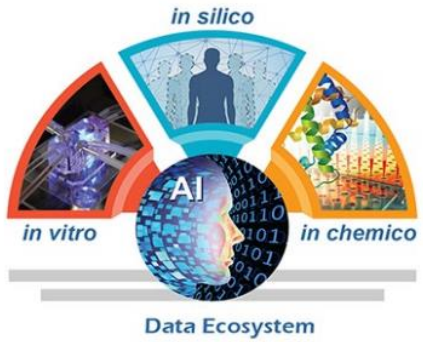
- ➢ The adoption by the pharmaceutical industry and regulators has been relatively slow
- ➢ Qualification for specific contexts of use (COU) supporting reproducibility and efficacy is necessary
- ➢ Disclosure of assay development for specific COU would be supportive but difficult in pharma

Needs:

- ➢ Collaboration between consortia and regulatory bodies is essential to for integration of CIVM/MPS

On **January 25, 2024**, The National Institutes of Health (NIH) Council of Councils approved the concept of the new NIH Common Fund's Complement Animal Research In Experimentation (**Complement-ARIE**) Program to speed the development, standardization, validation, and use of human-based New Approach Methodologies (NAMs).
[Complement Animal Research In Experimentation (Complement-ARIE) Program | NIH Common Fund]

Complement-ARIE: Innovate, Integrate, Coordinate, and Transform



- **Innovate** understanding of human health and disease pathways across diverse populations
- **Integrate** innovative NAMs (*in vitro, in chemico, and in silico*) with AI and FAIR data ecosystems
- **Coordinate** with ICs, agencies, and public-private partnerships
- **Transform** the way we do basic, translational, and clinical sciences by leveraging the full scientific toolbox

NIH National Institutes of Health
Office of Strategic Coordination–The Common Fund

Projected 10-Year Timeline and Budget

2025	2026	2027	2028	2029	2030	2031	2032	2033	2034
$35M	$35M	$40M	$40M	$40M	$40M	$40M	$40M	$40M	$40M

- ***Technology development projects/centers -*** *$23 – 26M per year*
- ***Data & NAM coordinating center -*** *$5 – 6M per year*
- ***Validation network for regulatory implementation -*** *$3 – 4M per year*
- ***Community engagement and training -*** *$3 – 4M per year*
- ***Strategic engagement-*** *$1 – 2M per year*
- ***RMS: NIH staff salary, travel -*** *$1M per year*

NIH National Institutes of Health
Office of Strategic Coordination–The Common Fund

Slides and figure from NIH


MPS WORLD SUMMIT
CONNECT, EXCHANGE, EDUCATE
June 10-14, 2024 | Seattle, Washington

Acknowledgements

DAIICHI SANKYO CO., LTD.

- Drug Metabolism and Pharmacokinetics Research Laboratories
- Medicinal Safety Research Laboratories
- Discovery Research Laboratories

One global team

DAIICHI SANKYO EUROPE GmbH

- Tissue and Cell Research Center Munich