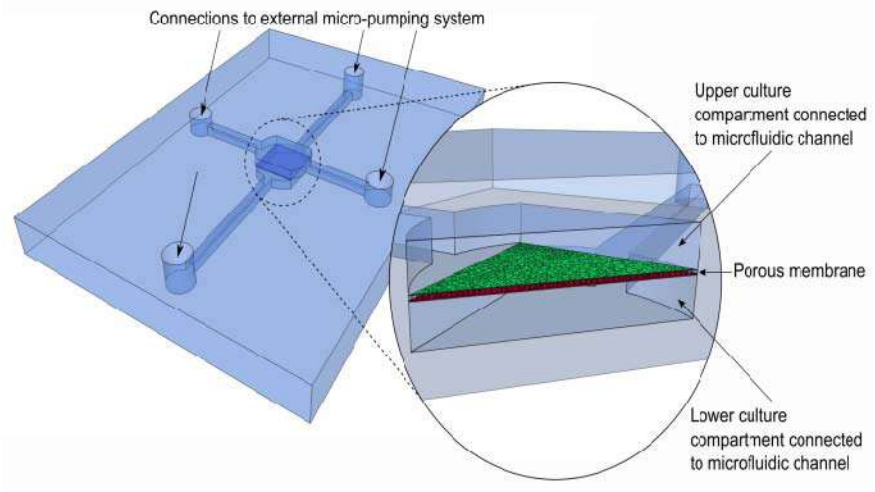
# Novel in vitro human model systems for immune-mediated diseases

*Example: auto-immune vasculitis*

*Anja van de Stolpe, Robert Kauffmann*

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# Immune-mediated diseases “auto-immune diseases”

- The problem:
  - The immune system of animals is different from that in humans:
    - Highly human
    - Different depending on genomic background (e.g. copy number variations)
    - Determined in part by geographical location: selection of specific immune genome
  - animal models for auto-immune diseases not representative for human disease
  - **Minimal progress in drug development**

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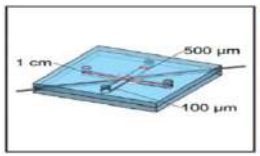
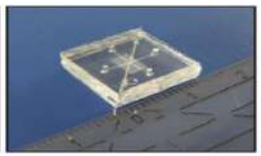
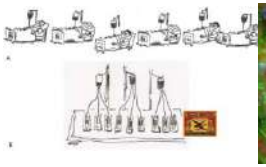
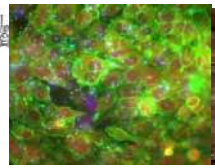
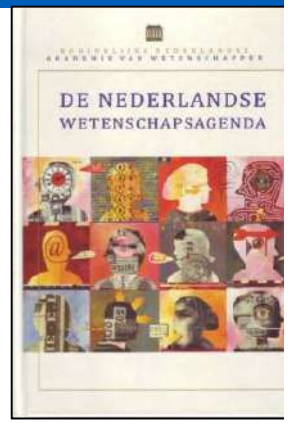
**High need for human in vitro model systems for immune-mediated diseases**






# Institute for *h*uman Organ and Disease Model Technologies : *h*DMT

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# What is organ-on-chip?

Culture the smallest functional modules of healthy or diseased tissues or organs on a microfluidic “chip”

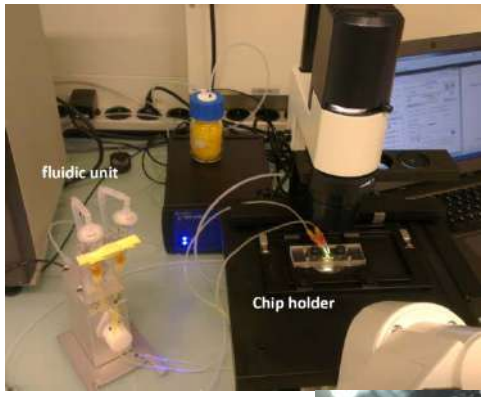
Highly multidisciplinary: stem cell technology, microfluidics, microelectronics and microfabrication

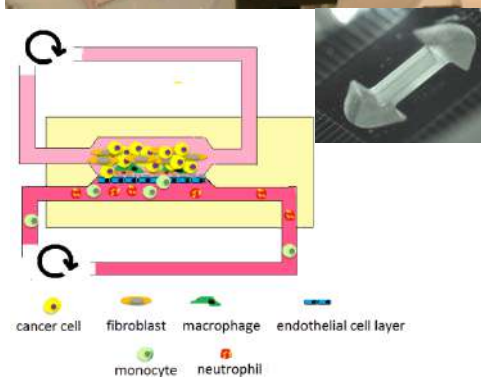
Cell culture

Past 50 years…….

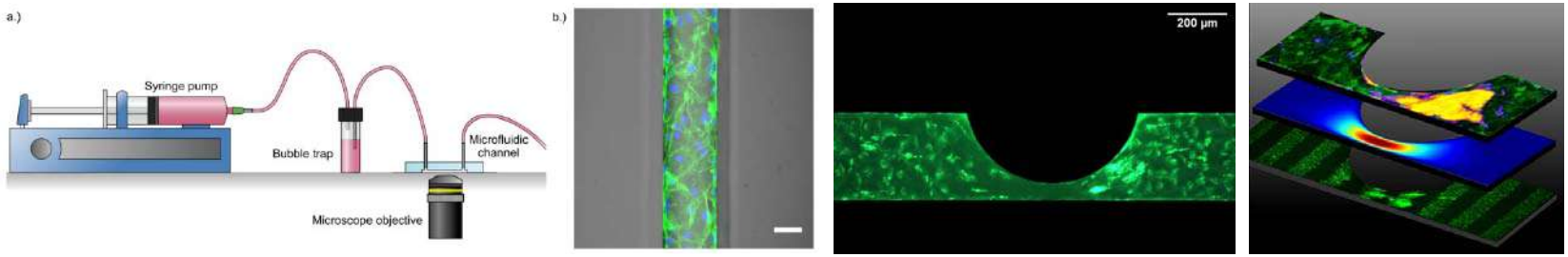
- 3D culture
- Different cell types, including immune cells
- **(Patho)physiological environment: biochemical + physical factors**
- **Long term culture: time for disease to develop**
- **Real time monitoring**
- Human stem cells from healthy individuals and patients: **human genetics**

Organ-on-chip

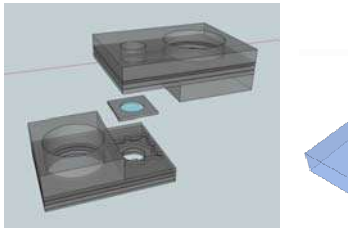


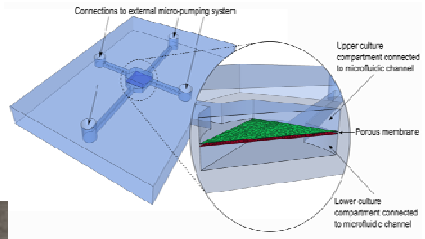



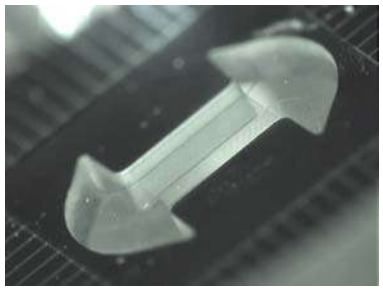
# Example organ-on-chip models

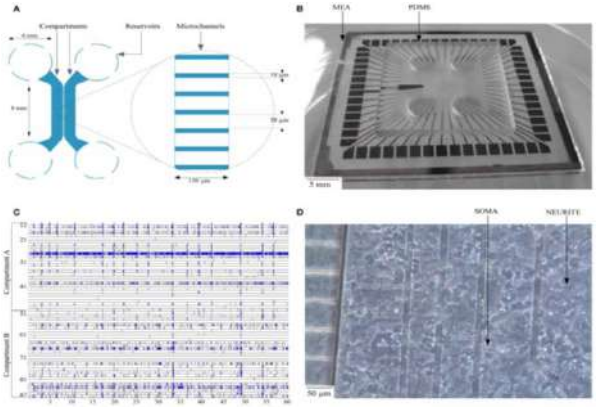


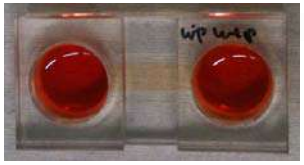

**Microfluidic chip for Human model for atherosclerosis and coagulation activation (Albert van den Berg, Utwente)**

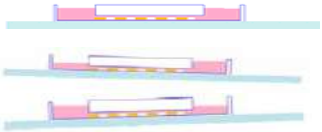










**Dual culture chamber chips with passive or active perfusion (Philips Research)**

**Chip with flexible electrodes for heart on a chip (Philips Research/TUDElft)**

**Neuronal network chip (Philips Research)**

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# Dutch Institute for *h*uman Organ and Disease Model Technologies

## *Our Research Program*

*Computational modeling*

1. Disease models
    1. Cardiovascular diseases (blood vessel, heart)

    2. Cancer: towards immunocompetent primary tissue organoid culture-based cancer-on-chip models

    3. Exploratory:
        - Neurological diseases (neuronal network)
        - **Immune-mediated (e.g. diabetes, IBD, vasculitis, fibrosis)**

2. Models for Lifestyle applications
    1. Skin and Hair
    2. Microbiome

3. Toxicity models
    1. Heart; blood-brain barrier; placenta; kidney

# Auto-immune vasculitis

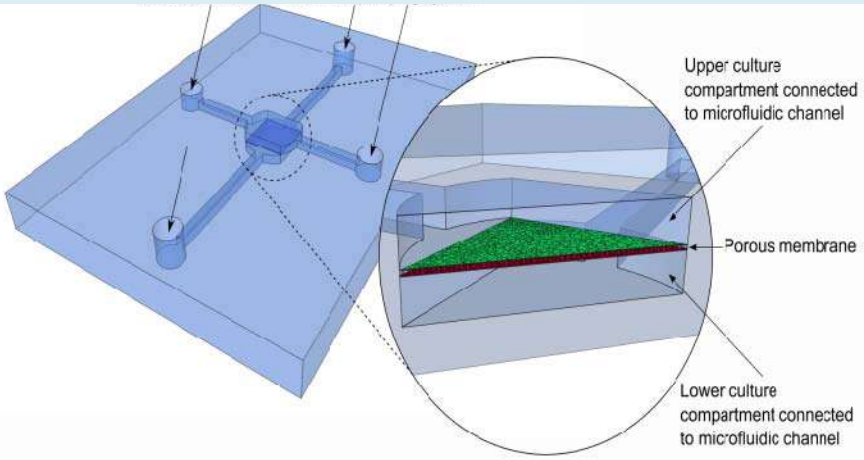
*Clinical questions:*

- Pathogenesis and pathophysiology:
  - Which genetic variations in combination with environmental factors influence clinical and histopathology presentation, including organ location, progression and response to therapy?
  - What are the autoimmune mechanisms that cause loss of tolerance?
  - What is the mechanism behind endothelial inflammation?
  - Self antigens (MPO, PR3 and LAMP or others): their pathogenic role play? Mechanism? Type of ANCA (IgG, IgM, IgA)?
  - What is the role of immune complexes and complement activation?
- Diagnosis: Can a better diagnostic classification be developed based on genetics and immunopathology?
- Disease monitoring: Which biomarkers can be used to reliably monitor disease status and therapy response?
- Drug development: Can disease-specific drug targets be found for drug development, for example to induce local resolution of the auto-inflammatory process?

# Organ on chip model for immune-mediated vasculitis

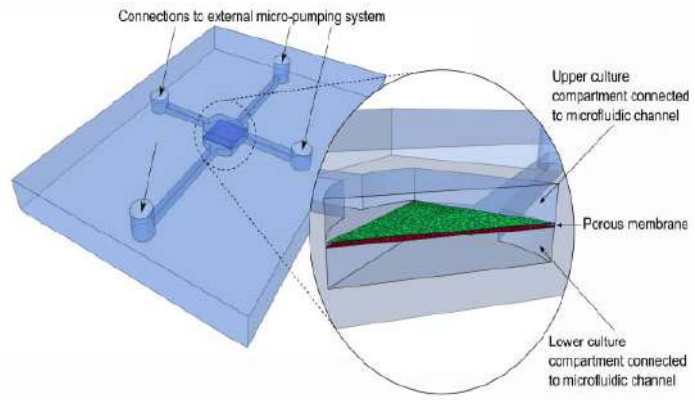
What do we need?

1. Dual chamber Chip
2. Cells:
    1. Human organ-specific endothelial cells in culture
    2. Immune cells
    3. Target organ tissue
3. Readout
4. Computational disease model



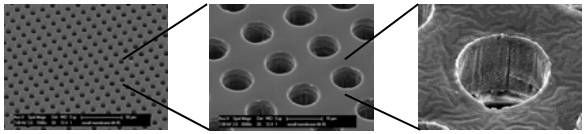

HUMAN ORGAN AND DISEASE MODEL TECHNOLOGIES

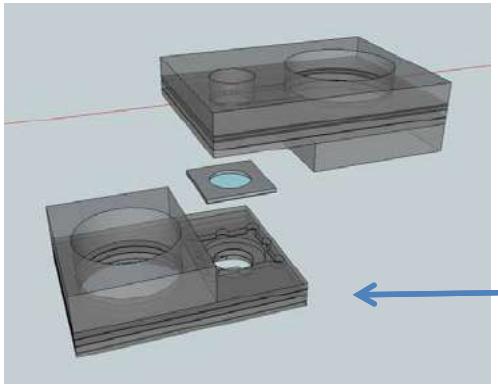
# Organ on chip model for immune-mediated vasculitis



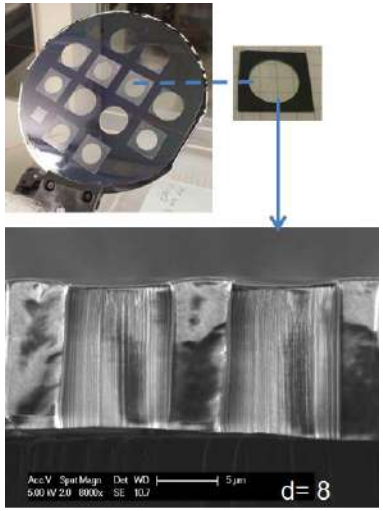

## Dual compartment microfluidic chip

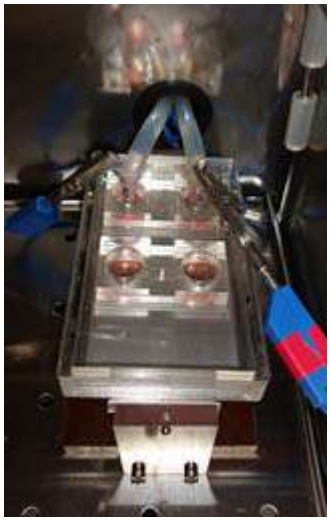
*Containing the target disease tissue, for example lung tissue, and an endothelial cell layer*



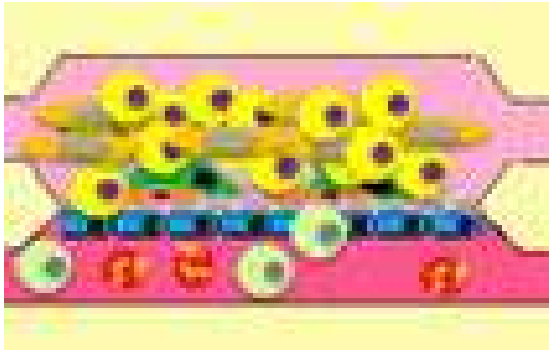


Separated by a porous membrane……



Measuring TEER



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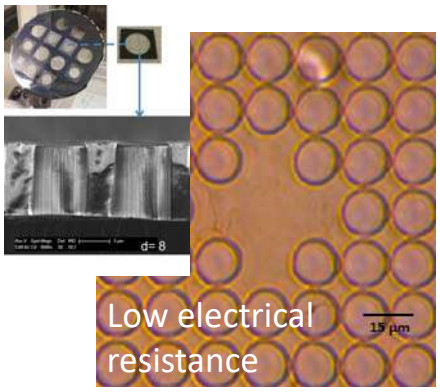
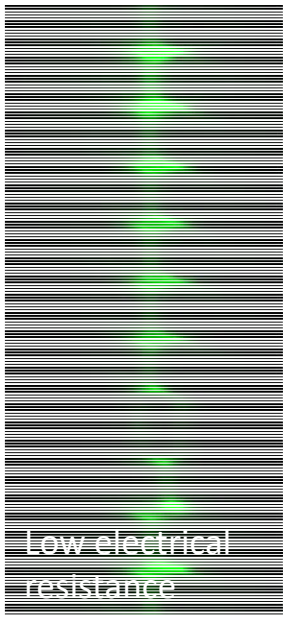
# Organ on chip model for immune-mediated vasculitis

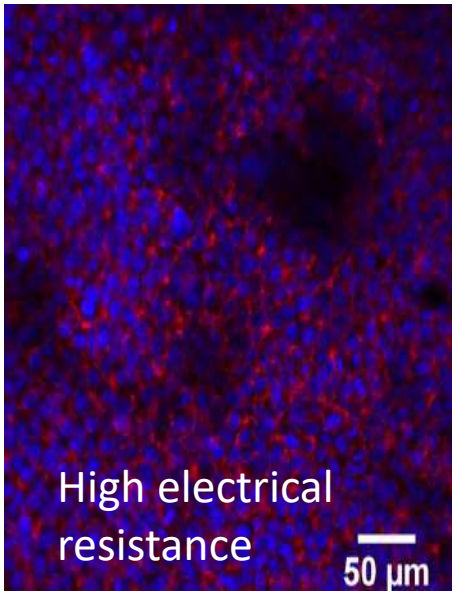
*Growing a confluent cell layer on a microporous membrane*



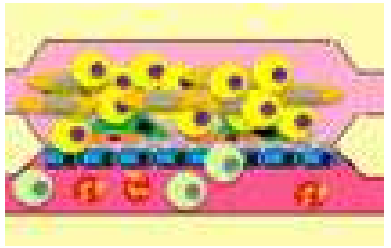

Delft ZOOM porous membrane, (*Zeer Open Organ-on-chip Membrane*)

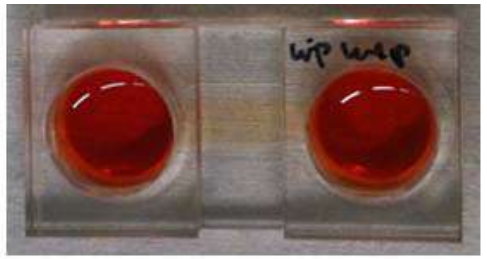


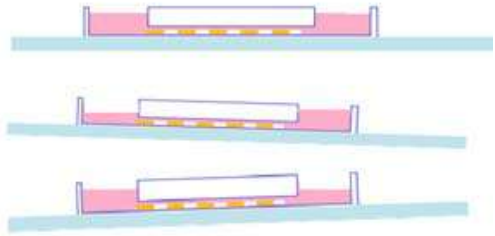

Microporous membrane filled with degradable gel




Confluent HEK293T cell layer





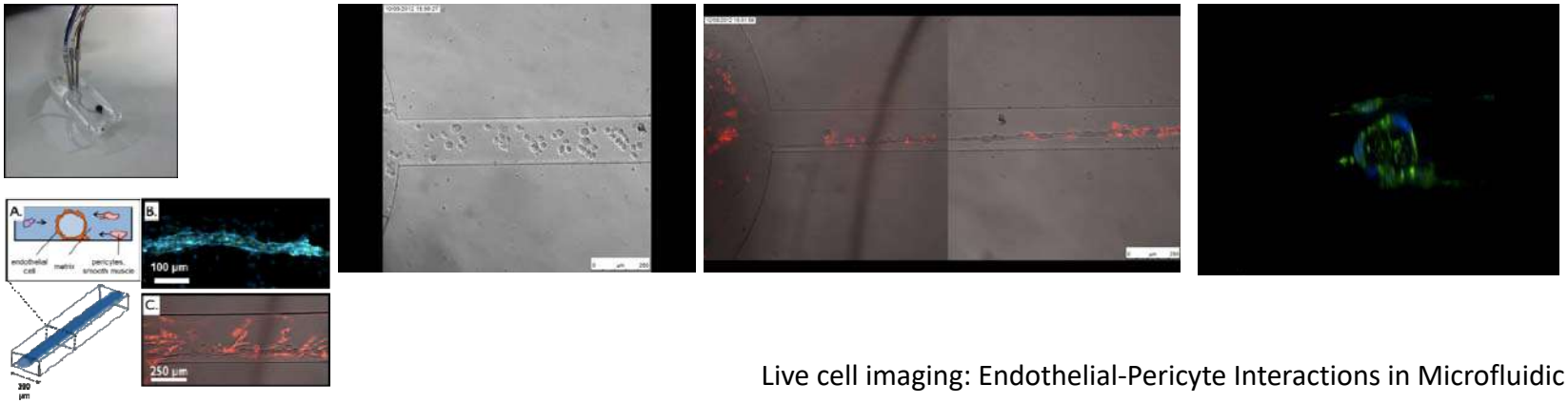
*Wip-wap perfusion device*

*Marlie van Lier, Lambert Bergers, Philips Research*

# Organ on chip model for immune-mediated vasculitis

*Human organ-specific endothelial cells in culture: iPS cell derived*

**Human blood vessel-on-chip** (Valeria Orlova, Andries van der Meer, Albert van den Berg, Christine Mummery) LUMC/UTwente




Live cell imaging: Endothelial-Pericyte Interactions in Microfluidic Channels

Readouts:

- biomarkers that can be measured real time using fluorescence and a microscope, or measured in culture fluid
- Pathology analysis

HUMAN ORGAN AND DISEASE MODEL TECHNOLOGIES

# Organ on chip model for immune-mediated vasculitis

*Which immune tissue should be incorporated in a disease model on-a-chip?*

*Important: for adaptive immune response: HLA compatible with other tissue in culture model*

**Innate immune response:**

- **Primary** cells or cell lines + iPS-derived tissue culture

**Adaptive immune response:**

- **iPS-derived** immune cells/tissue + tissue culture *from the same individual*
- **Primary immune cells** of a patient + HLA matched iPS cell line derived cells/tissue

1. Inflammatory response

**Tissue/organ located** dendritic cells

**Circulating cells** (monocytes, neutrophils)

2. Adaptive immune response

**Peripheral lymphoid organs**

- **Lymph node**

3. Development of immune response/tolerance

**Primary lymphoid organs**

- **Thymus**
- **Bone marrow**

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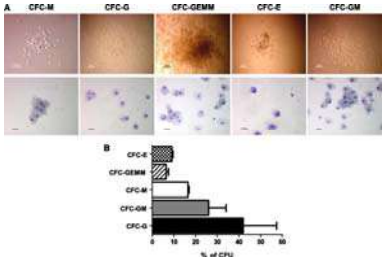
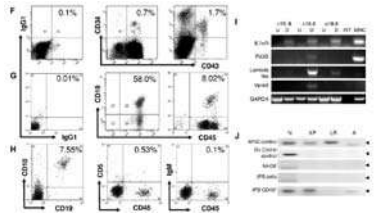
# iPS-derived immune cells/tissue: 3D tissue architecture




**All blood cell progenitors, including monocytes, macrophages:**
Kim E et al. Blood 2013.
*Zavazava, University of Iowa*
(Van Wildenburg, Plos One, 2013;
*Cowley, Oxford)*



**B-lymphocytes:**
Carpenter, Blood. 2011
*Suzanne Watt, Oxford*

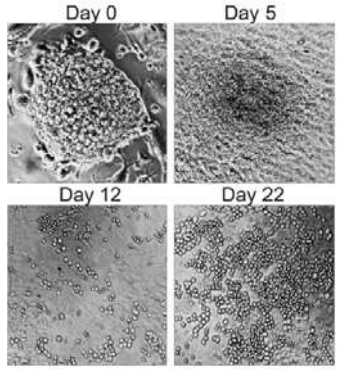



Figure 1.
Morphology of iPS cell differentiation. At various days, mouse iPS cells were co-cultured with OP9-DL1 cells in α-MEM medium supplemented with 20% FCS and 2.2 g/L sodium bicarbonate in the presence of 5 ng/ml mFltL and 1 ng/ml mIL-7

**T-lymphocytes :**
Lei. J Vis Exp 2012
*Song, Pensylvania State*

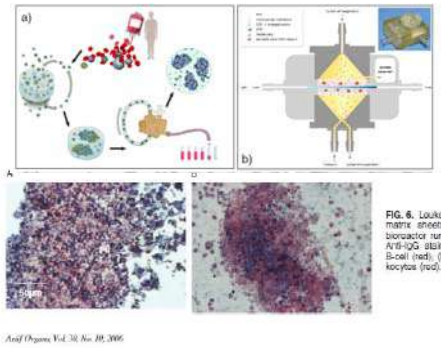
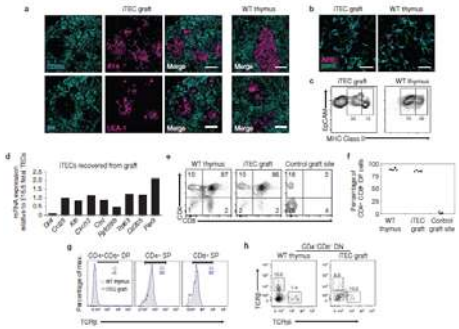


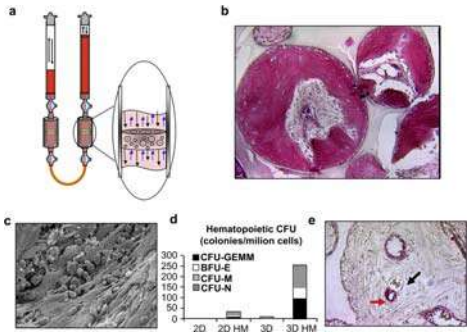

FIG. 6. Leukocyte clusters in a... matrix sheets, derived from a ... bioreactor run (hematoxylin staining). (A) Anti-IgG staining of a cluster indicating B-cell (red). (B) Anti-CMV staining ... leukocytes (red).

*Artif Organs Vol 30, No 10, 2006*

**In vitro Lymph node:**
Giese, Advanced drug delivery Reviews, 2014;
Giese, Biotechnol. 2010
*Marx, Technical University Berlin*




**In vitro Thymic tissue**
Bredenkamp, Nature Cell Biology, 2014
*Clare Blackburn, Edinburgh*




**In vitro Bone Marrow:**
DiMaggio 2014.
*Martin, Basel University*
Torisawa, Nature Methods, 2014
*Ingber, Harvard*

HUMAN ORGAN AND DISEASE MODEL TECHNOLOGIES

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*Important: for adaptive immune response: HLA compatible with other tissue in culture model*

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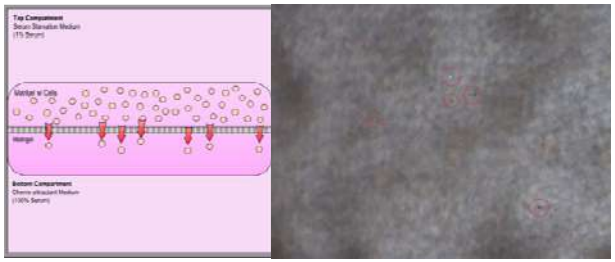

**Adaptive immune response:**

- **iPS-derived** immune cells/tissue + tissue culture *from the same individual*
- **Primary immune cells** of a patient + HLA matched iPS cell line derived cells/tissue

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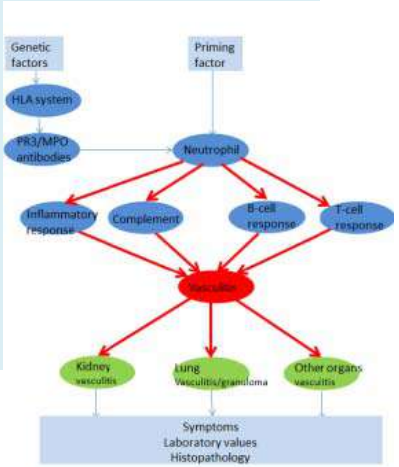
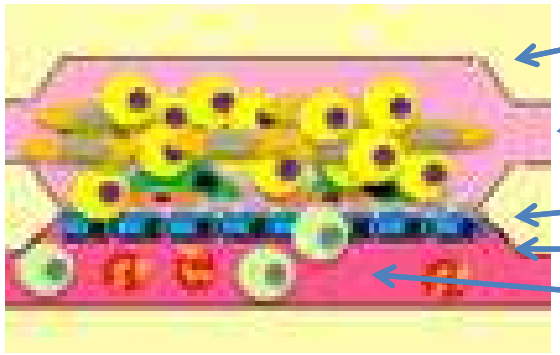
readout




Computational model



chip

membrane

confluent endothelial cell layer

migrating monocytes (cell line)

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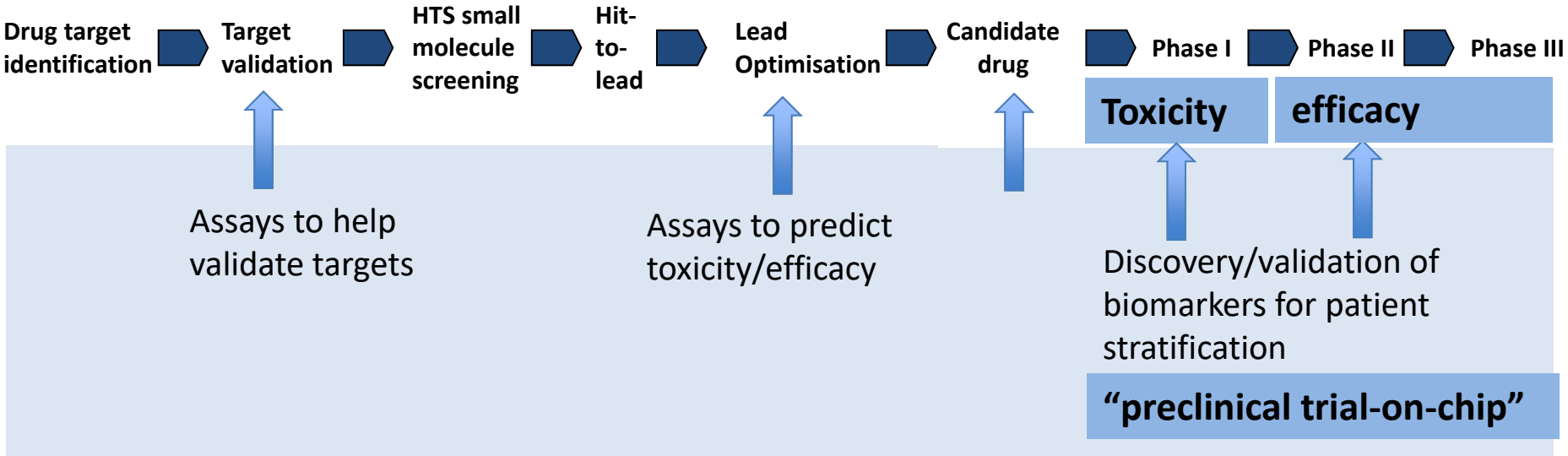
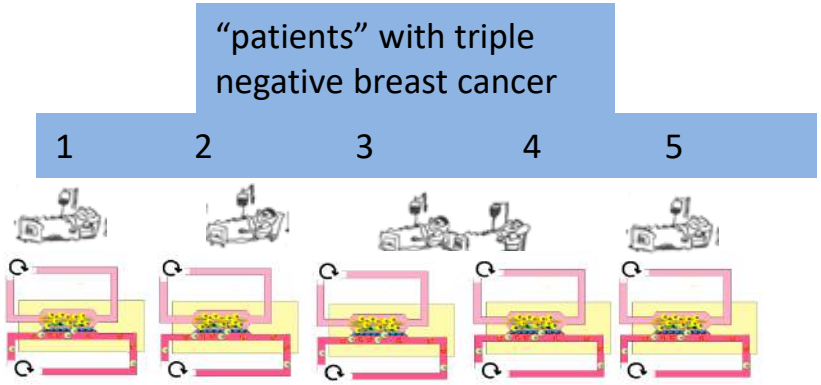
# Where does organ-on-chip fit in the drug/treatment development process – *including companion diagnostics*?




Human organ-on-chip disease model:

- Pathology validation
- Multiple human genetic variants
- Low throughput testing




**“preclinical trial-on-chip”**

- with multiple “patients” on a chip
- Organoid + iPS-based culture: build an increasing number of storable and reusable “patients-on-chip”. *In contrast to real patients!*

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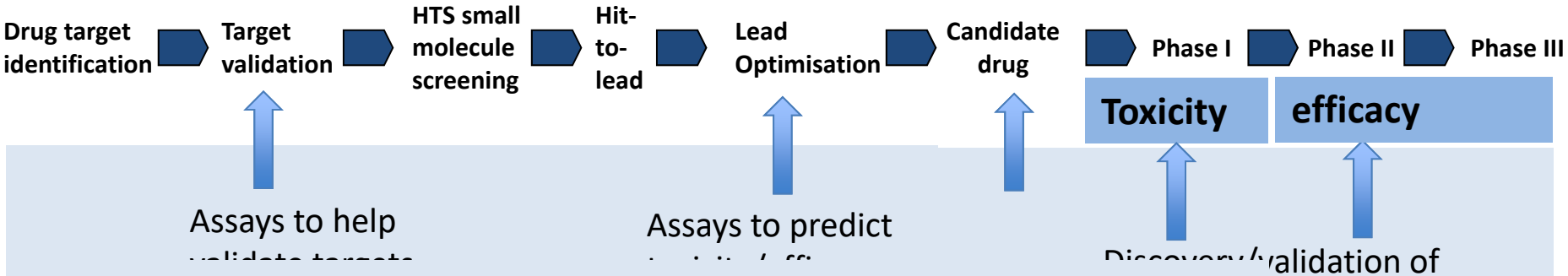
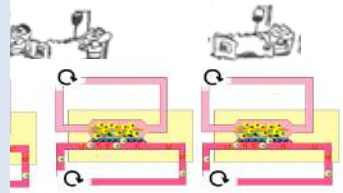
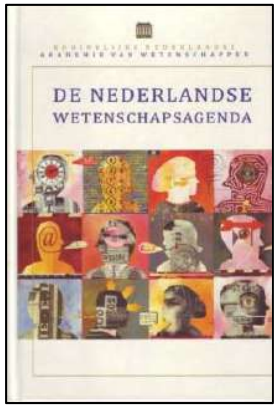



- Speed up drug development
- Reduce costs
- Decrease late stage failures in clinical trials
- Off-label drug testing
- **Orphan diseases**
- ***Reduce animal experiments***



HUMAN ORGAN AND DISEASE MODEL TECHNOLOGIES




# Acknowledgement

**All consortium members who contributed to hDMT** (especially the core team including Christine Mummery, Albert van de Berg, Lina Sarro, Richard Janssen)

**Janny van den Eijnden and Mieke Schutte, managing directors hDMT**

Royal Netherlands Academy of Sciences and Technical University Delft for sponsoring the Startup phase

**Cancer-on-a-chip**

Philips Research
- Roland Vulders
- Alwin Verschueren
- Freek van Hemert
- Lambert Bergers (VUMC)

Students
- Emiel van Genderen
- Marlie Lier
- Tom van Gijsel (Now Philips Research)
- Erik van Buitenen
- Francesca Rivella