ProBioGen

Intelligent Biopharmaceutical Solutions

HUMANE SOCIETY INTERNATIONAL

Modelling Human Immunity in Health and Disease

Human Artificial Lymph Node Model (HuALN)
for Biopharmaceuticals Testing and Disease Modelling in vitro

BioMed21, Brussels (Dec 8-9, 2015)

C. Giese and A. Lubitz

Content

🔬 **Challenges of modelling human immunity *in vitro***

🔬 **Recent status on relevant *in vivo* models
and current achievements for *in vitro* modelling**

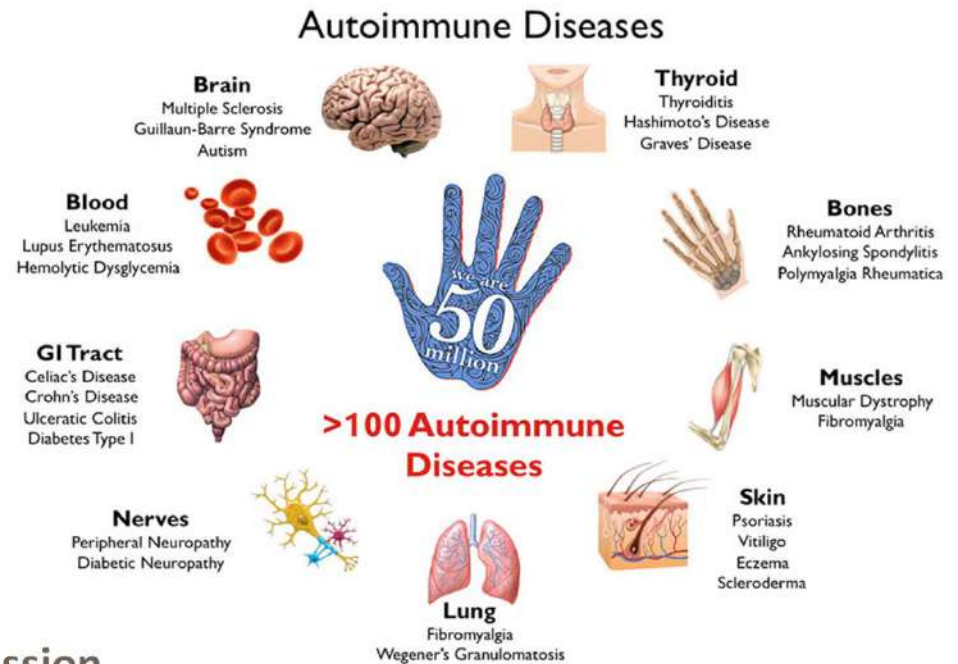🔬 **The HuALN model**

🔬 **Conclusions**

Modelling Autoimmune Diseases

Innovative human-specific investigational approaches to autoimmune disease
(Anja van de Stolpe and Robert H. Kauffmann RSC Adv. 2015 (5))

**Pathogenesis of autoimmune diseases:
Breaking tolerance**

Source: www.cytherapharm.com

Therapeutical intervention:
- "Old school" treatment: Immune suppression
- Innovative therapy: Inducing tolerance

Modelling the Human Immune System

Understanding immunity:

★ Development and organogenesis

★ Homeostasis, regeneration and aging

★ Immune responses
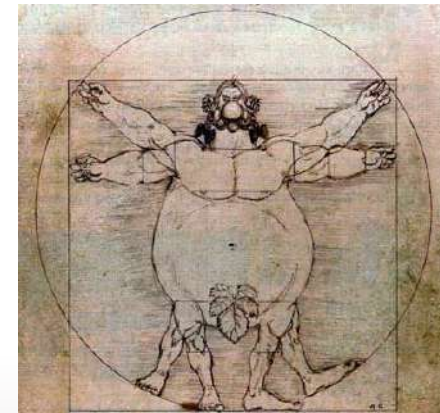
★ Immune system-related diseases

Treating immunity:

★ Vaccination (Inducing immunity)

★ Infection diseases (Breaking tolerance)

★ Cancer (Lymphoma, leukemia; improving immunity)

★ Inflammation diseases (Inducing tolerance)

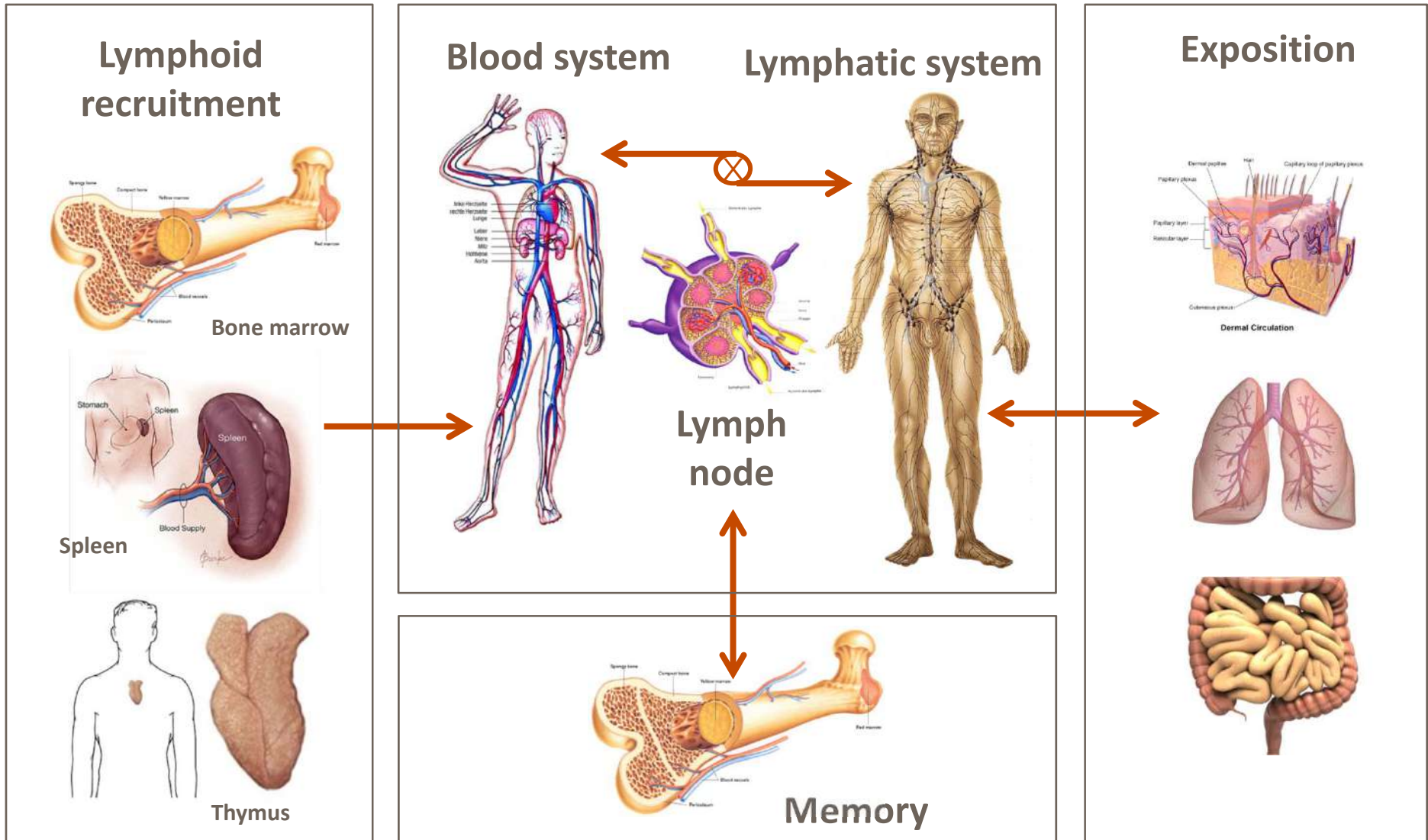★ Allergy and sensitization (Inducing tolerance)

Hhealth
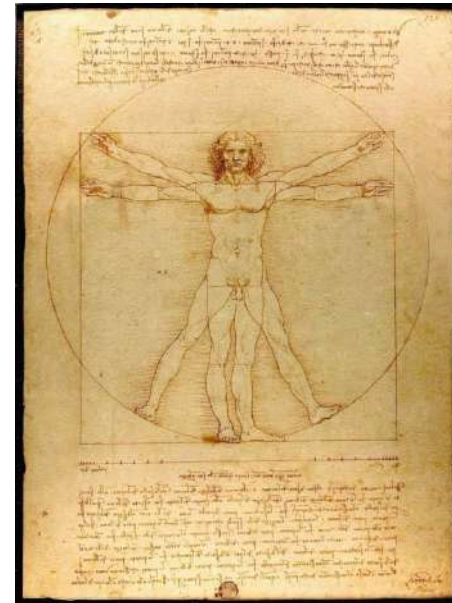
The Vitruvian Man
(Leonardo da Vici, approx. 1490)

Disease

Source: www.comedix.de

The Complexity of the Human Immune System

Lymphoid recruitment

Bone marrow

Spleen

Thymus

Blood system

Lymphatic system

Lymph node

Exposition

Memory

Micro Physiological Systems (MPS) for the Human Immune System

Biological challenges and technical solutions for MPS

- Solid organs
  (Tissue complexity, cellular composition, compartmentalization)
- Mobile cells and fluidics
- Innate and adaptive, cellular and humoral responses
- Systemic effects
- Lifelong memory

Challenges for MPS by biological heterogeneity

- Population heterogeneity
- Seasonal changes, previous infections, hidden therapeutical treatments
- Non responders
- Diseases and disease status
- Maturation, age and senescence

Biological Scaling

Minaturization and „scalability of immunity"

What is the minimum functional unit of an organ?

Liver

Organ → Liver lobuli

Function

Albumin production
Cytochrome activity
Bile production
...

Immune system

Primary /secondary/tertiary immune organs

Critical T cell and B cell repertoire?

Technical environment

Function

Innate response
Cellular responses
Humoral responses
Immunity/memory
Cytokine release
Antibody production
Cellular reactivity
...

In vivo Testing: Customized Animal Models

Wildtype and chimeric
targeted transgenic mice

- ✦ **Immune deficient mice** (e.g. SCID)

- ✦ **Transgenic mice** (Vector based; gene knock-out/knock-in)

- ✦ **Humanized mice** (with human immune cells)

- ✦ **Xenograft models** (with human tumor cells)
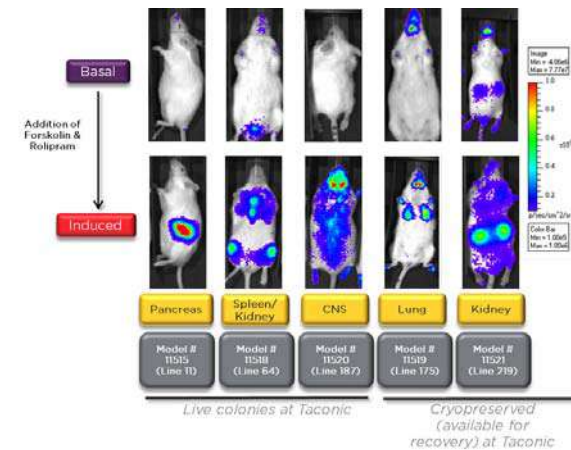
- ✦ **Genome-edited animals** (CRISPR/CAS9)?
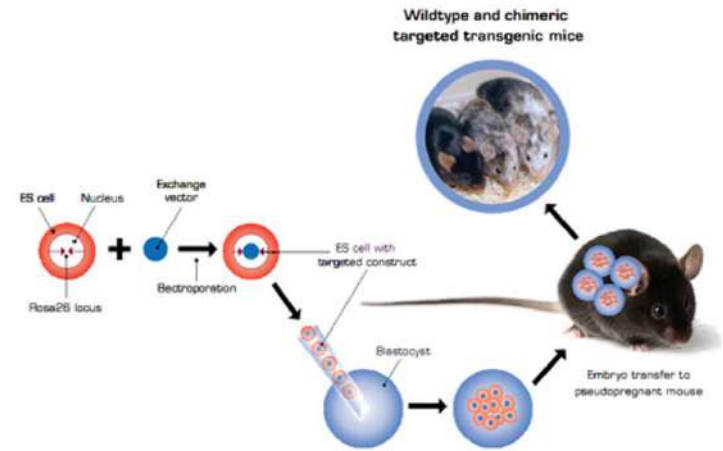
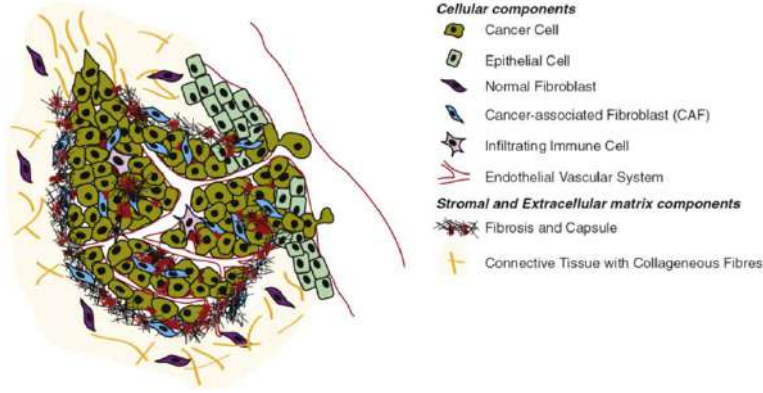**Transgenic mice model**
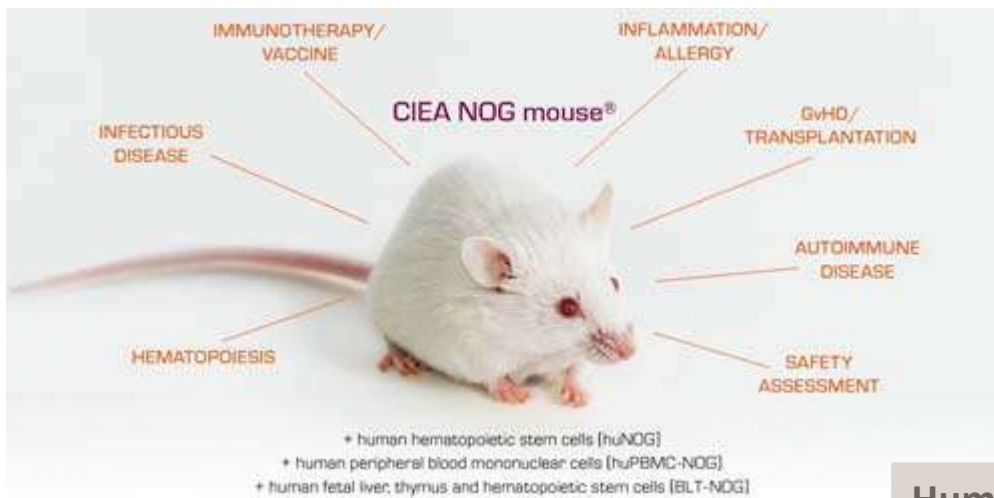
Humanised mice

Humanized animals as well a xenograft models show limitations in reproducibility and relevance to a certain extent.

Spheroid Tumor Models
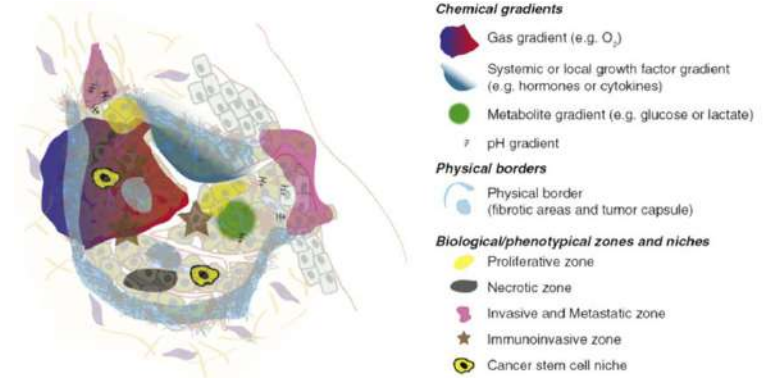
The „cellular suspects"

Cellular components
- Cancer Cell
- Epithelial Cell
- Normal Fibroblast
- Cancer-associated Fibroblast (CAF)
- Infiltrating Immune Cell
- Endothelial Vascular System

Stromal and Extracellular matrix components
- Fibrosis and Capsule
- Connective Tissue with Collageneous Fibres

The micro-enviroment

Chemical gradients
- Gas gradient (e.g. O_2)
- Systemic or local growth factor gradient (e.g. hormones or cytokines)
- Metabolite gradient (e.g. glucose or lactate)
- pH gradient

Physical borders
- Physical border (fibrotic areas and tumor capsule)

Biological/phenotypical zones and niches
- Proliferative zone
- Necrotic zone
- Invasive and Metastatic zone
- Immunoinvasive zone
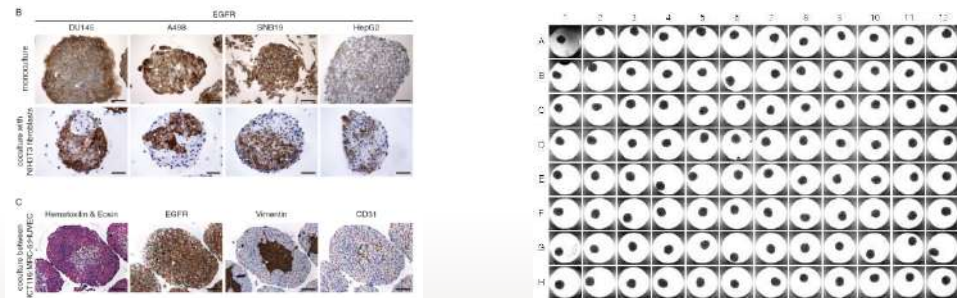- Cancer stem cell niche

Multi-cell-type 3D tumor micro-spheroid co-culture models (hanging drop technology)

In combinations with cancer cells endothelial cells and stromal cells
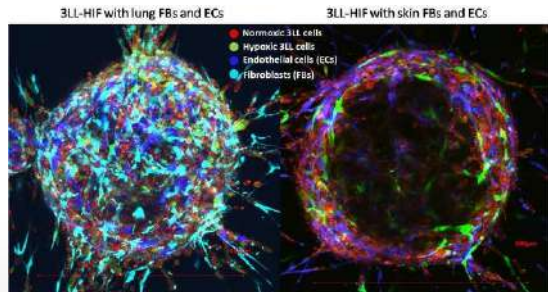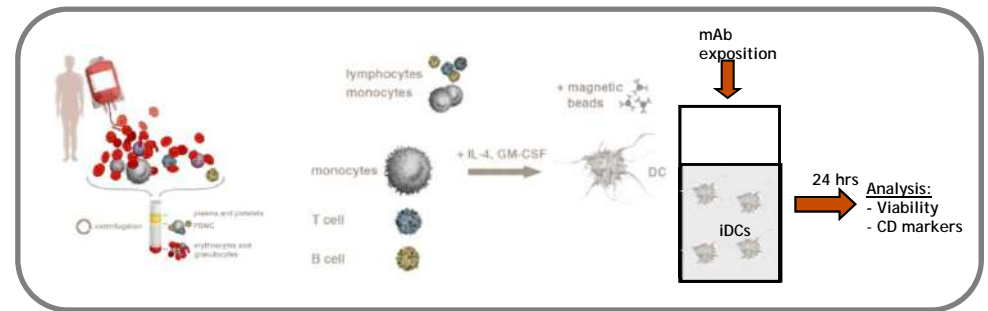

Source: Volgin etal (2010)
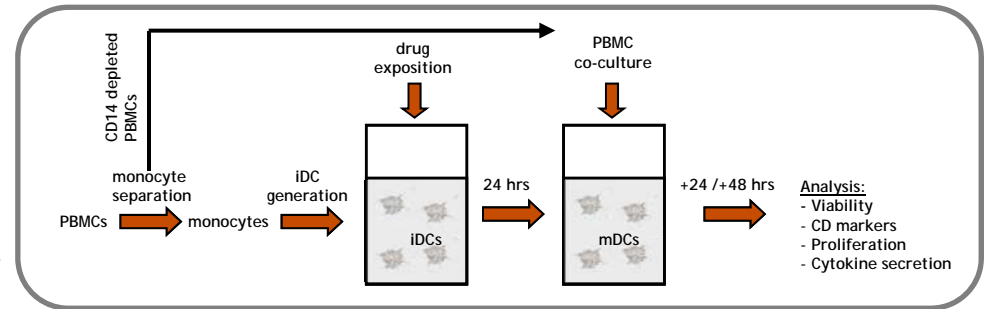
Source: Kelm etal (ADDR, 2014)

Cell-based *in vitro* Methods for Immune Functional Testing at PBG

- ADCC, ADCP

- Peptide binding assays

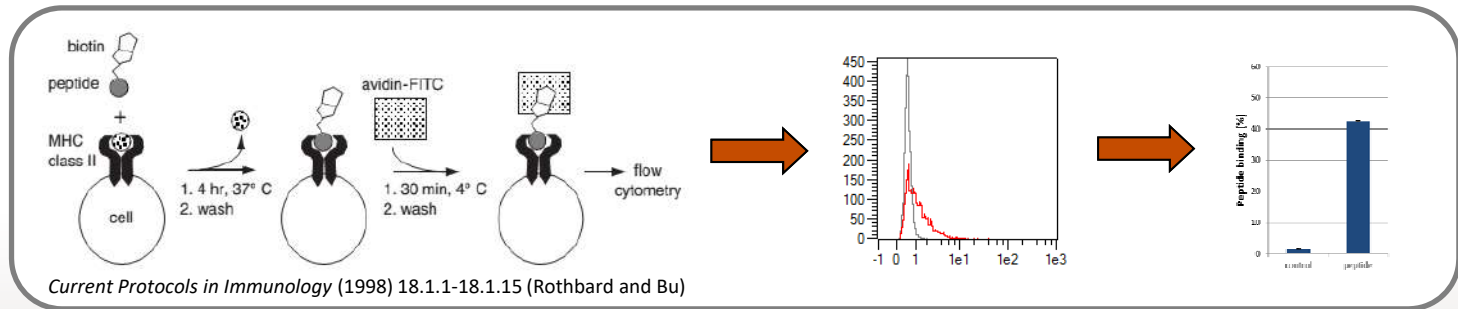- PMBC's testing
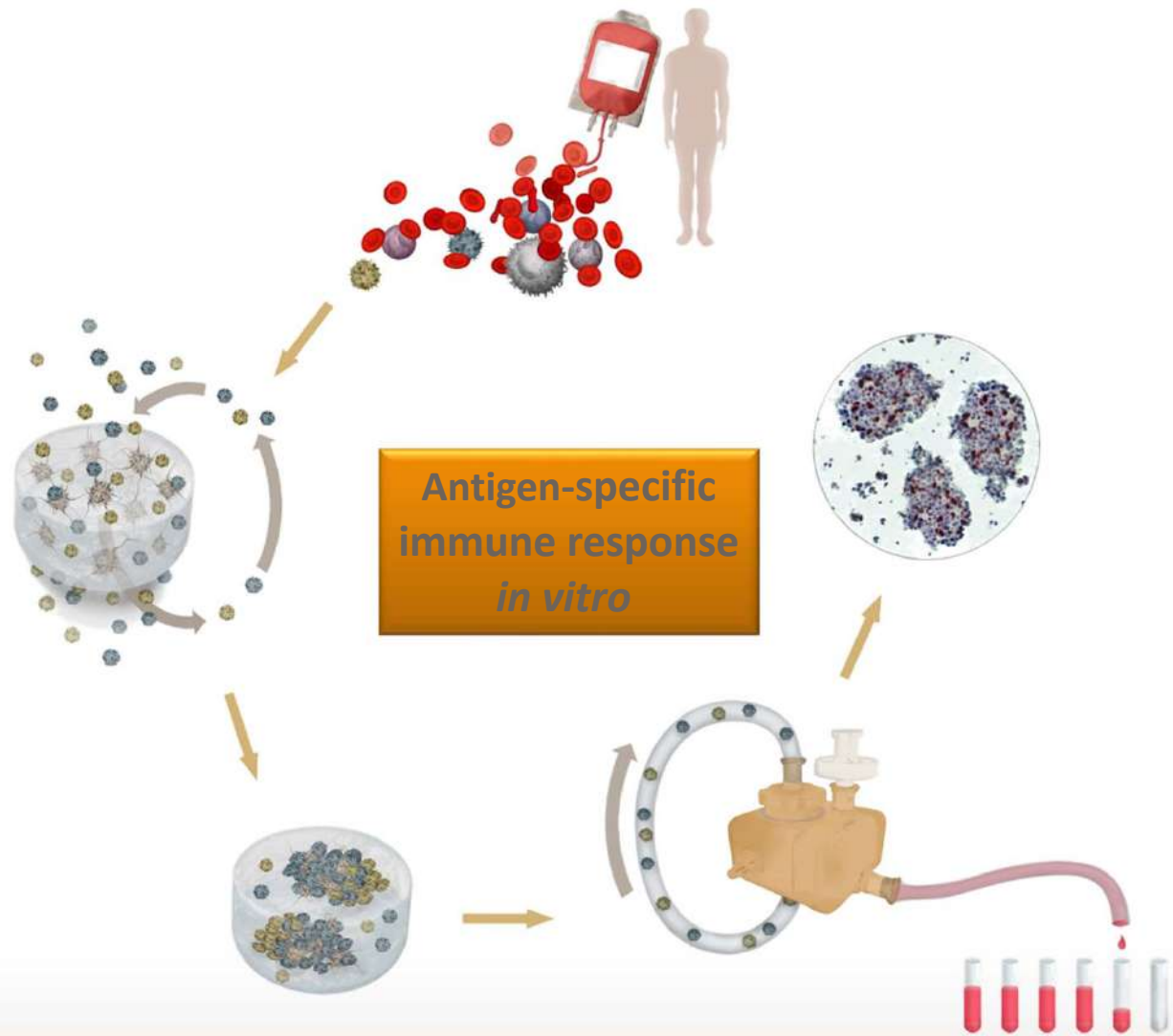
- DC and DC/T cell assays

- HuALN Model

DC assay

mAb exposition

24 hrs — Analysis:
- Viability
- CD markers

iDCs

DC/T cell assay

CD14 depleted PBMCs

drug exposition   PBMC co-culture

monocyte separation   iDC generation   24 hrs   +24 /+48 hrs   Analysis:
PBMCs → monocytes →   iDCs →   mDCs →   - Viability
- CD markers
- Proliferation
- Cytokine secretion

Peptide binding assay
MHC I/II
human mDCs
FACS

Current Protocols in Immunology (1998) 18.1.1-18.1.15 (Rothbard and Bu)

The Human Artificial Lymph Node Model (HuALN)

Antigen-specific immune response *in vitro*

The Human Artificial Lymph Node Model (HuALN)
Testing Services in Four Applications

IG

Immunogenicity

VAC

IF

Vaccine testing

ITOX

Immunofunctional testing

Immunotoxicity
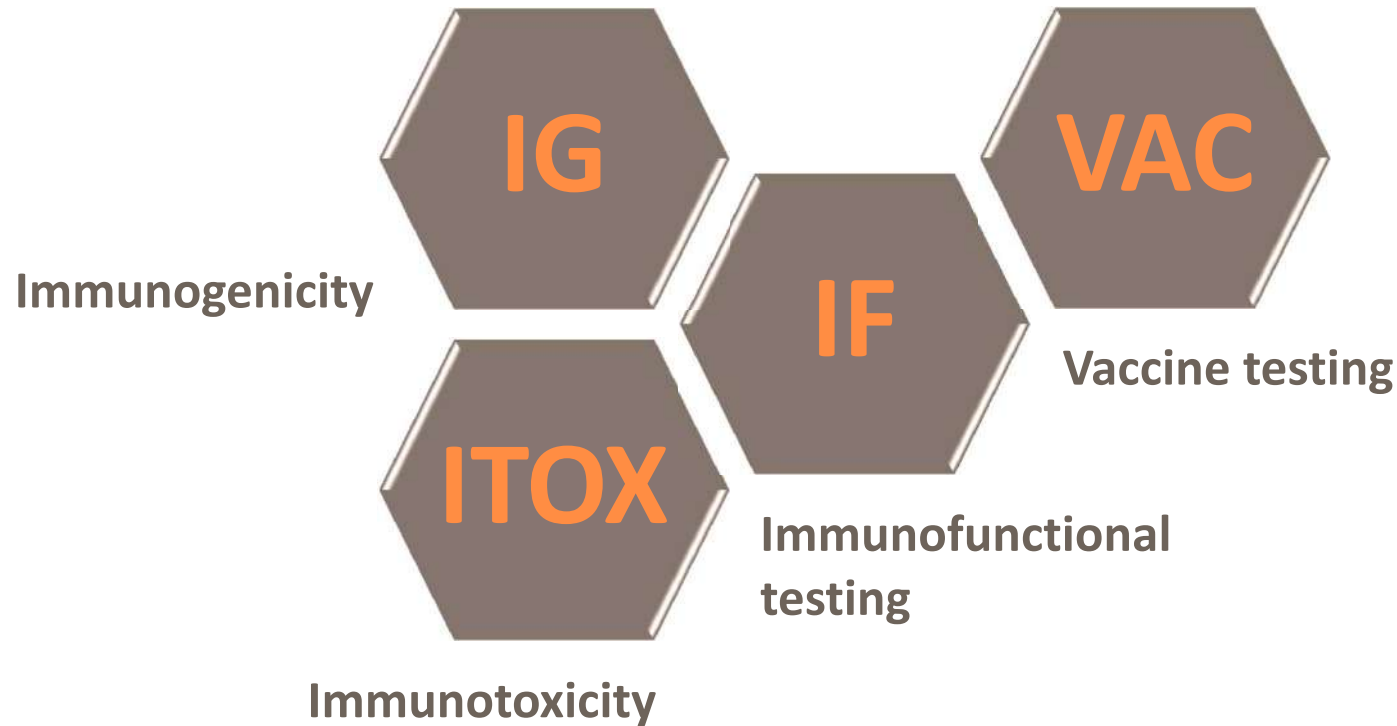
IG: Understanding unwanted immunogenicity of biopharmaceuticals and formulations
IF: MoA and adverse effects of super-agonists and checkpoint modulators
VAC: Potency, candidates ranking, dosing and MoA of viral and peptide vaccines, adjuvants and formulations
ITOX: Assessing chemical immunotoxicology

Bioreactor Devices

HIRIS 3

2 mL culture volume (perfusion rate 1 mL/day)-
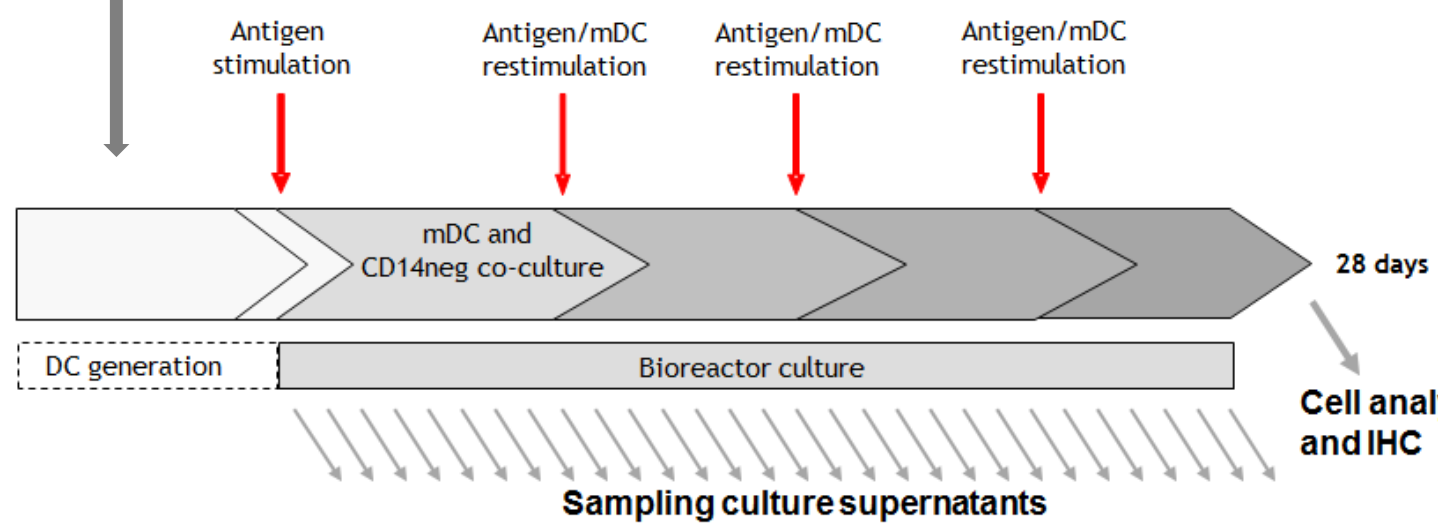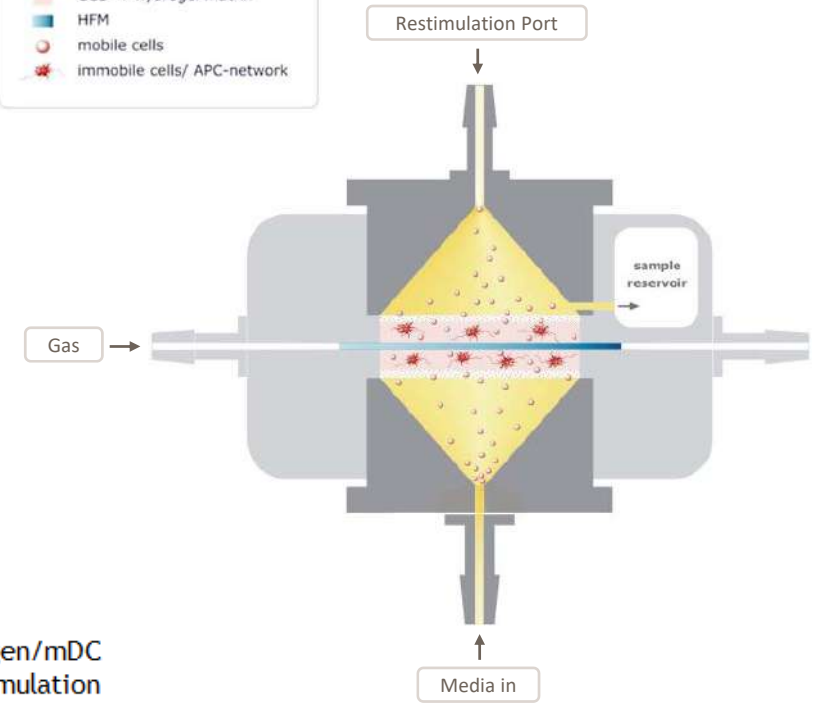large cell repertoire (10^8 DC/PBMC) -
cell perfusion -

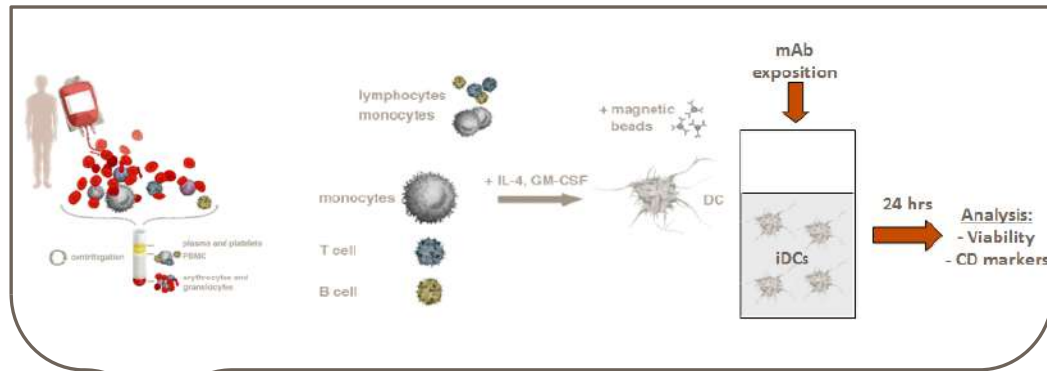
µALN (µALN 1.0)

- Miniaturized size (10-20 fold)
- 150-250 µL culture volume (perfusion rate 100 µL/day)
- Reduced cell repertoire (10^6-10^7 DC/PBMC)
- Parallelization for increased number of test
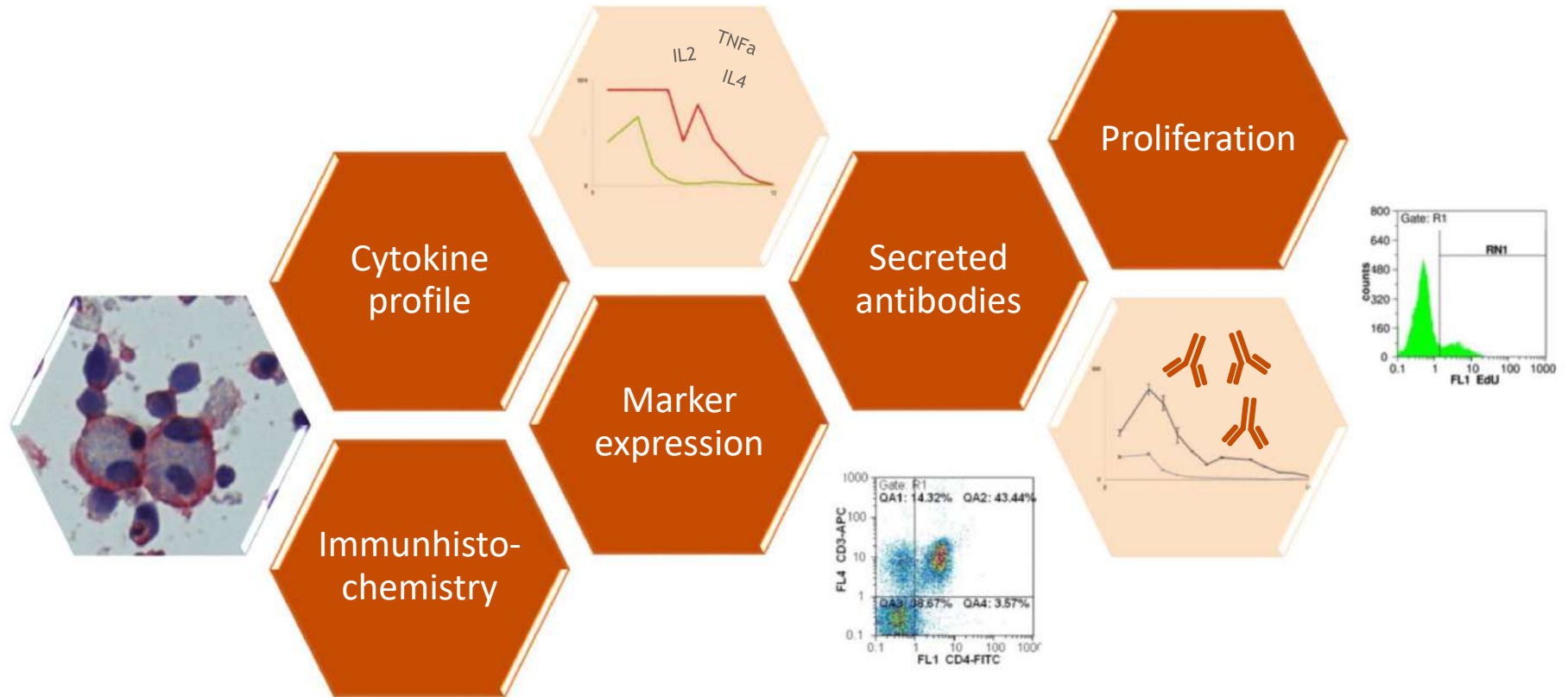 samples and screening applications
- User friendly
- *In-situ* imaging
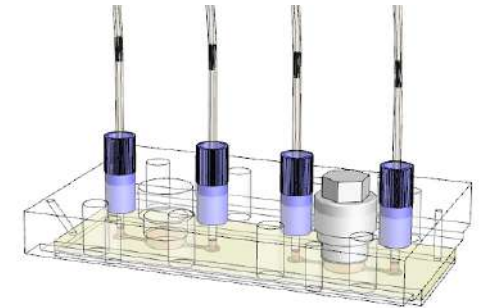
Basis for an autonomous device (µALN 2.0):
Integrated fluidics, heating and gassing
No incubator, no external fluidics, easy to handle

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Modelling Human Immunity in Health and Disease

The HIRIS Bioreactor Device -
Perfusion, Drug Exposition and Sampling

HuALN: Analytical Parameters

- Cytokine profile
- Immunhisto-chemistry
- Marker expression
- Secreted antibodies
- Proliferation

Monitoring Cytokines to Immune Response Analysis

T cell activation for TH-1 pathway

Hubbell, Thomas, and Swartz; NATURE 2009
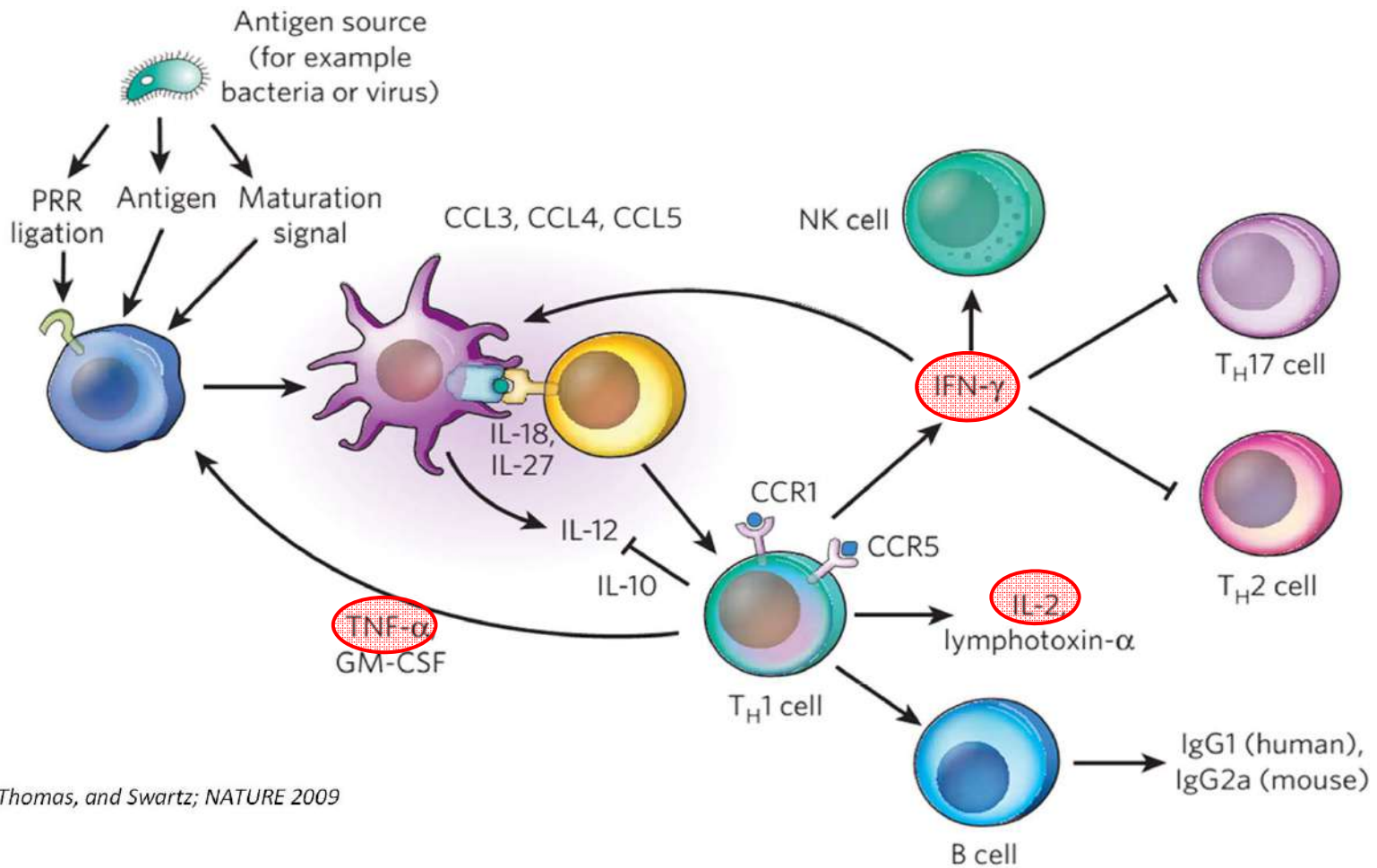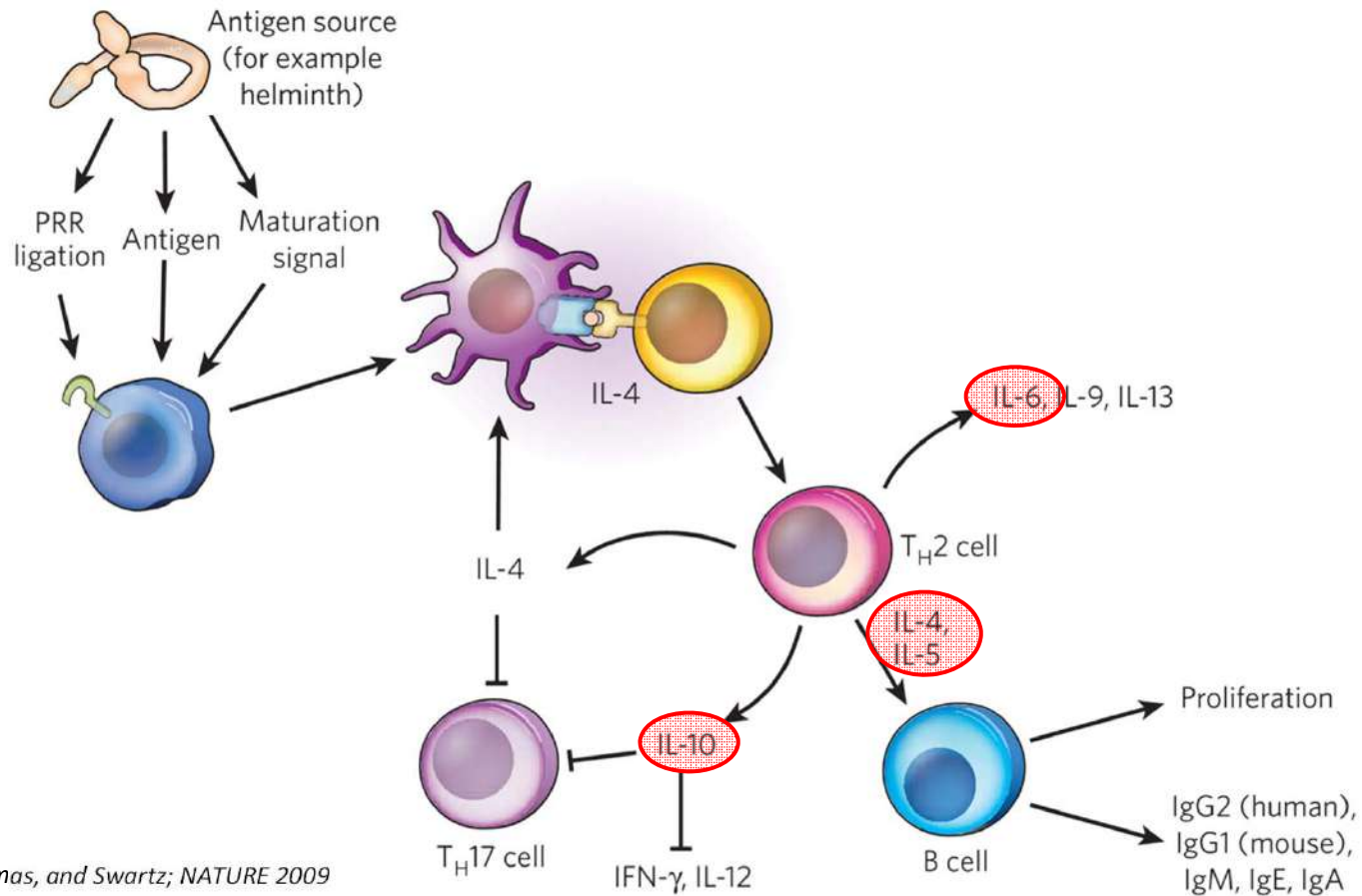
Monitoring Cytokines to Immune Response Analysis

T cell activation for TH-2 pathway

Hubbell, Thomas, and Swartz; NATURE 2009

Immune Responses Triggered by Cytokines
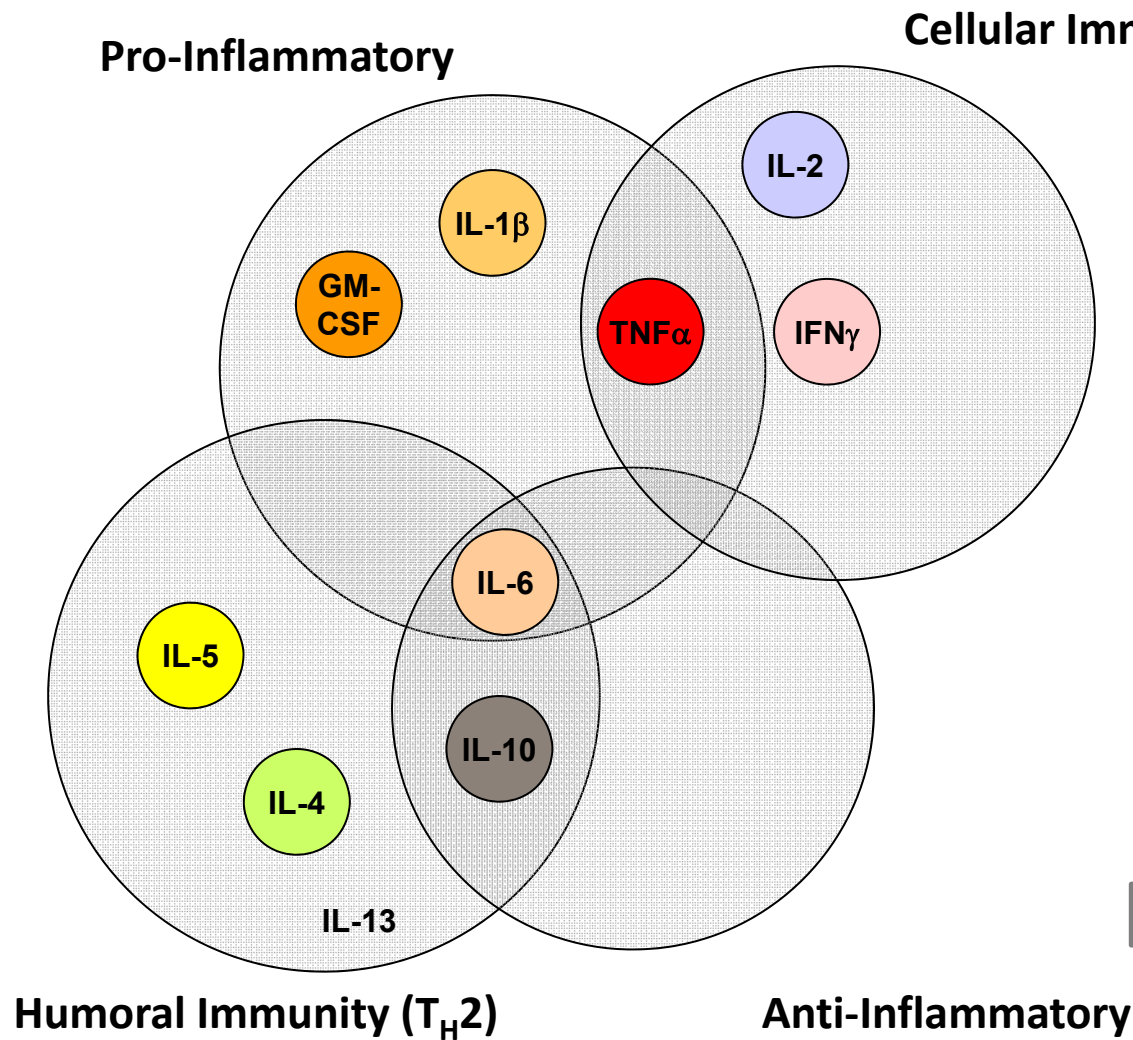
Cytokine groups

Group	Cytokines
Pro-inflammatory	IL-1ß TNFα IL-6 GM-CSF
Anti-inflammatory	IL-6 IL-10
T_H1	IL-2 IFNγ TNFα
T_H2	IL-4 IL-5 IL-6 IL-10

Normalized values of cytokines are added up complying the functional cytokine groups.

Graphical Cytokine Data Summary (Exemplified):
Immunogenicity of Induced Aggregates (Drug Substance: mAb)

Normalized to HSA (control)

Normalized to tox-buffer (control)

Cytokine groups (Classified by function)	
Pro-inflammatory	IL-1ß TNFα IL-6 GM-CSF
Anti-inflammatory	IL-6 IL-10
T_H1	IL-2 IFNγ TNFα
T_H2	IL-4 IL-5 IL-6 IL-10

Modelling Human Immunity in Health and Disease

19
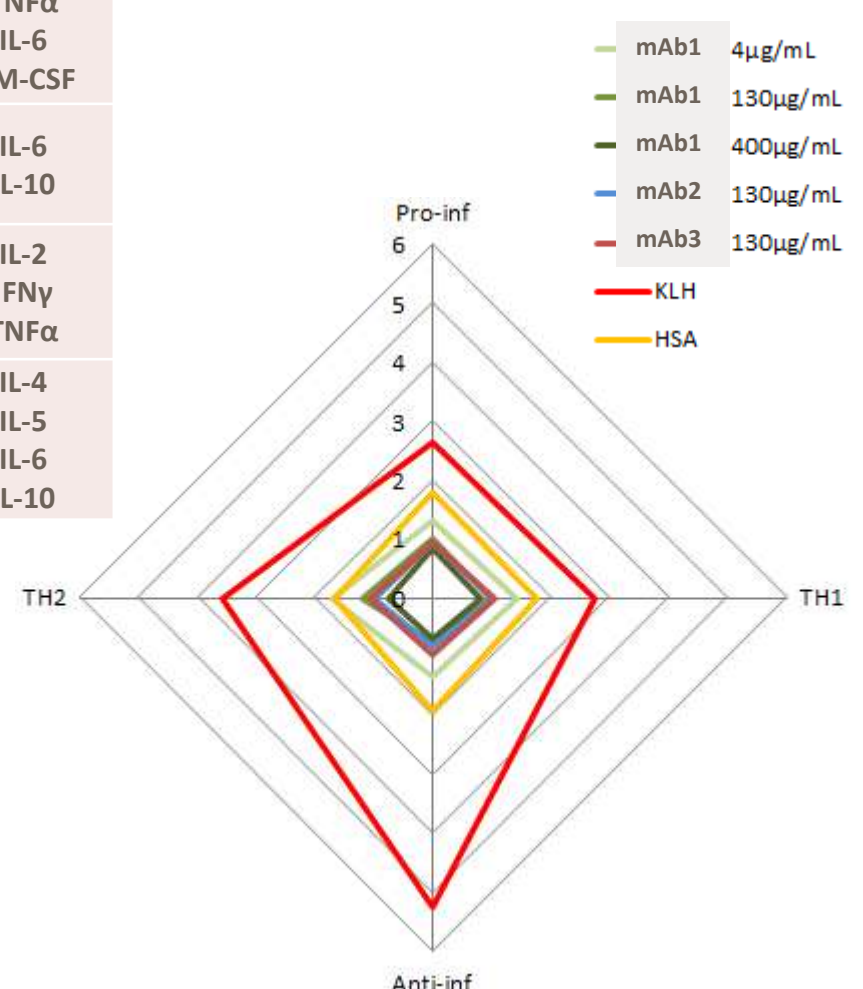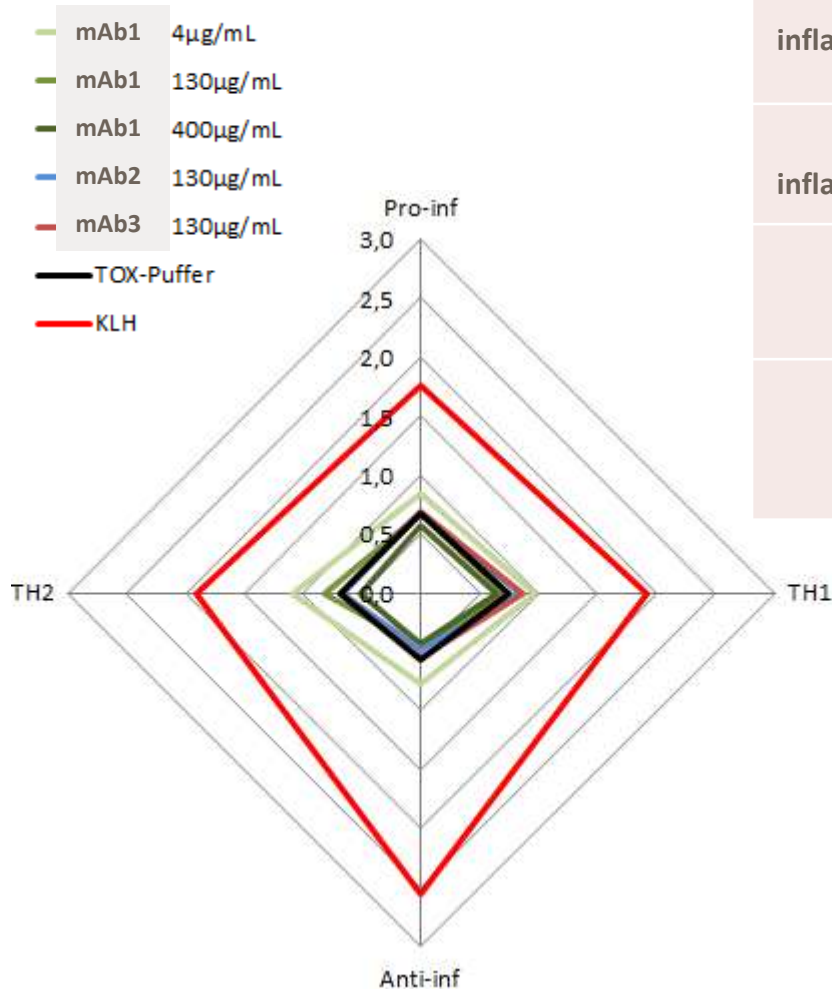
Aim: Human Disease Models in vitro
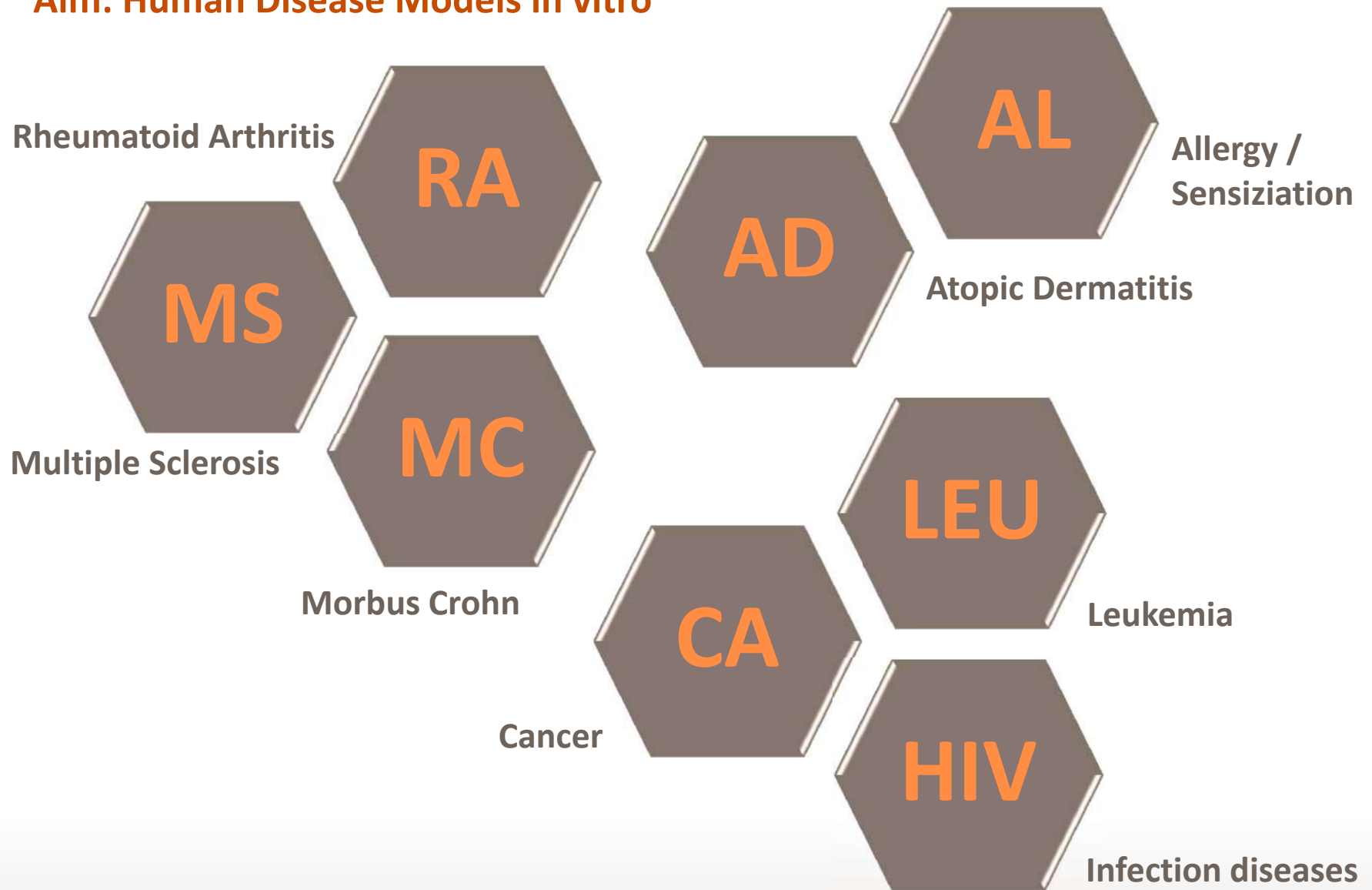
Rheumatoid Arthritis

RA

MS

Multiple Sclerosis

MC

Morbus Crohn

AD

AL

Allergy / Sensiziation

Atopic Dermatitis

LEU

Leukemia

CA

Cancer

HIV

Infection diseases

Modelling Autoimmune Diseases

Innovative human-specific investigational approaches to autoimmune disease
(Anja van de Stolpe and Robert H. Kauffmann RSC Adv. 2015 (5))

**Basic concept for modelling an inflammation disease
on a miniaturized organ model ("patient-on-a chip")**

Important achievements:
- **Miniaturized platform suitable for patient biopsy material**
- **Continuously perfused**
- **Long-term, organotypic culture**
- **Steady-state conditions**
- **Controlled drug exposition**
- **Dynamic response profiling**

Conclusions

In general:

🔸 **Good bioreactor platforms available (Single organ and MOCs)**

🔸 **Relevant human cell and tissue models available**

🔸 **Miniaturized and multiplexed analytics available**

🔸 **Advanced in situ-imaging technologies available**

HuALN:

🔸 **The HuALN model is a useful tool for testing immune human responses in vitro**
   Applications: IG, IF, Vac, Tox)

🔸 **The model is using "well trained" immune cells of adult and healthy donors**

🔸 **GLP-like test procedures available**
   (Medical devices, SOPs, fully documented, data/reports QA reviewed)

🔸 **The HuALN model is ready for service in Open for tech-transfer and licensing**

Outlook

In general:

- **Disease models with patient biopsy material**

- **Simulating disease models (Surrogates)**
    - **Drug induced inflammation**
    - **Leukemia/cancer (Co-cultivation models)**

- **Use of iPS technology and e.g. genome editing (CRISPR/CAS9)**

HuALN:

- **Spheroid model fir the immune system**

- **Inflamed HuALN model**

- **HuALN with patient biopsy material**

- **HuALN and tumor co-culture mode (Leukemia model)**