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Supporting Biopharmaceutical Visions

The human Artificial Lymph Node: A Model for Immunofunctional and Immunotoxicological Testing *in vitro*

Dr. Christoph Giese, ProBioGen AG

9th Annual *ecopa* Workshop
November 29-30, 2008, Brussels

Outline

- ProBioGen services

- The need for test methods *in vitro*

- The human ALN model:
  A new human tissue-like *in vitro* technology
  of predictive potential

ProBioGen Offers Cell-based Solutions for the Biopharmaceutical Industry

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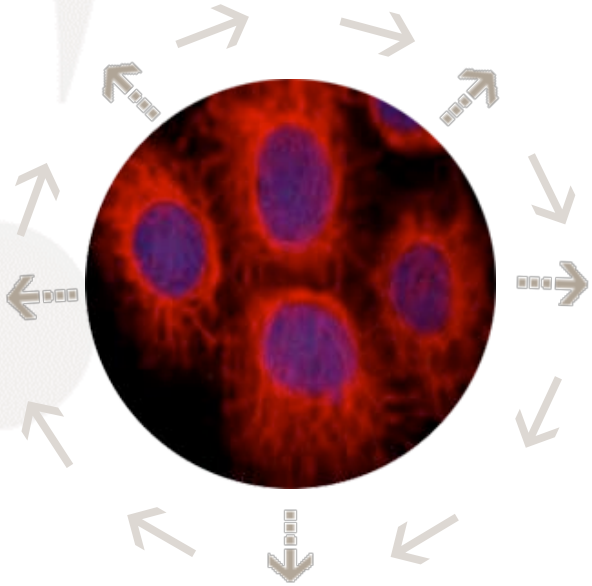
Bioassay Development
Analytical cell services

Cellular analytics & cell-based assays for product characterisation

Immunofunctional and immunotox testing using the proprietary *in vitro* model: **human Artificial Lymph Node**

GMP production
of clinical trial supplies

Services include the GMP **production** of biopharmaceutical drug substances **up to phase II**
Manufacturing authorisation (§ 13 AMG)
Quality standards required by the **EMEA** and the **FDA guidelines**
Strategic **alliance** with **Boehringer Ingelheim**



Cell biology
Sequence optimisation

Metabolic engineering to **increase** the specific **cell productivity**

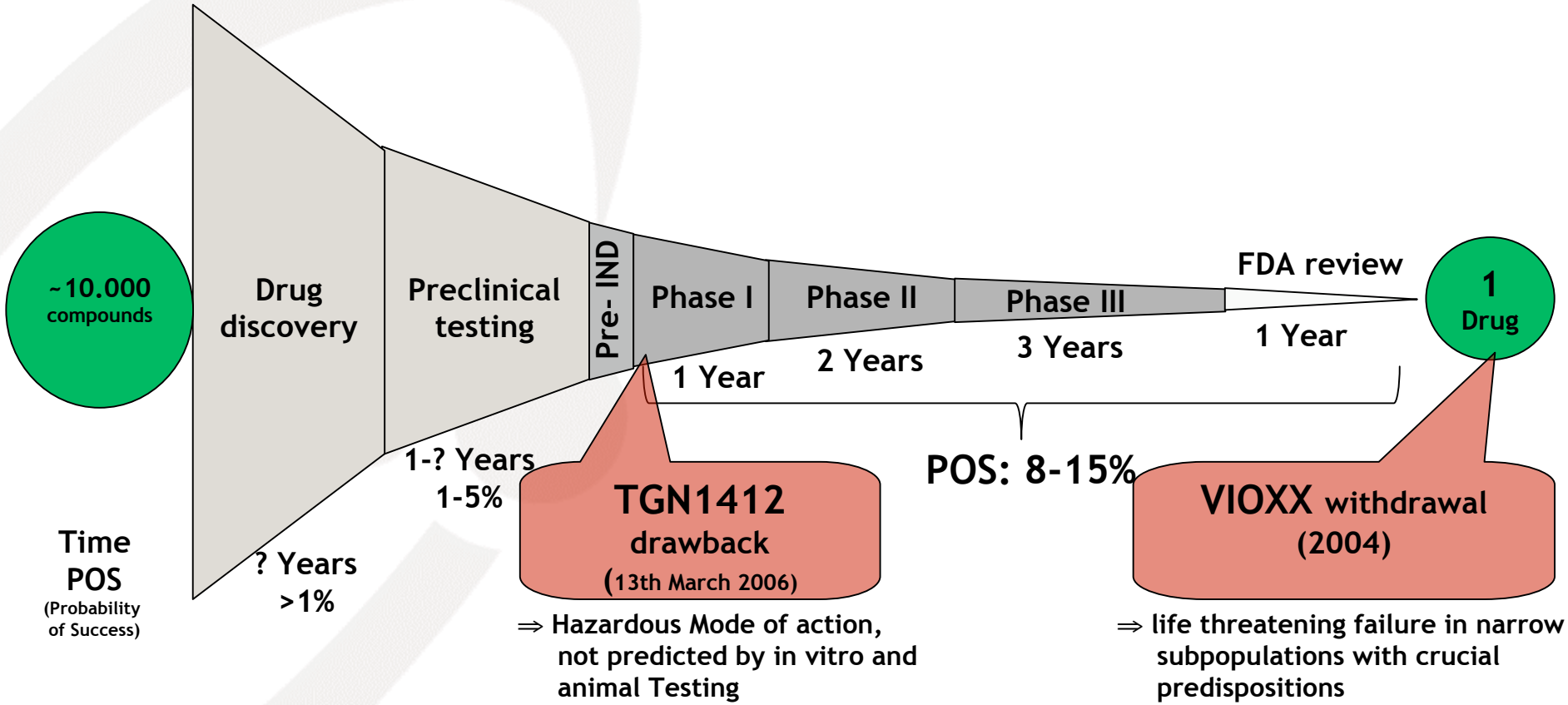
Cell line engineering

Development of **high-producer CHO** cell lines
Proprietary AGE1 cell lines for the manufacturing of vaccines and therapeutic proteins

Process engineering

Capacities **up to 1,000L**
Fully disposable manufacturing systems
Upstream / downstream
Cell banking

Challenges in Biopharmaceutical Candidate Development



Questions Related to the Product:

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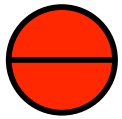
From Product Potency to Immunological Adverse Events in Patients

- Immune stimulation (e.g. IFN α/β)
- Immune suppression (e.g. corticosteroids, tacrolimus)
- Immunogenicity (of vaccines)
- Immune Tolerance (e.g. TRegs)
- Immune specific effects: ADCC, NK (e.g. MAbs)



- Adverse effects (e.g. TGN1412)
- Off-target effects (e.g. MAbs)
- Immunogenicity (NAb formation)
- Sensitization (e.g. cosmetics and compounds)
- Allergy and anaphylaxy

Cell Based Assays at ProBioGen for Predictive *in vitro* Testing of Immune Functions

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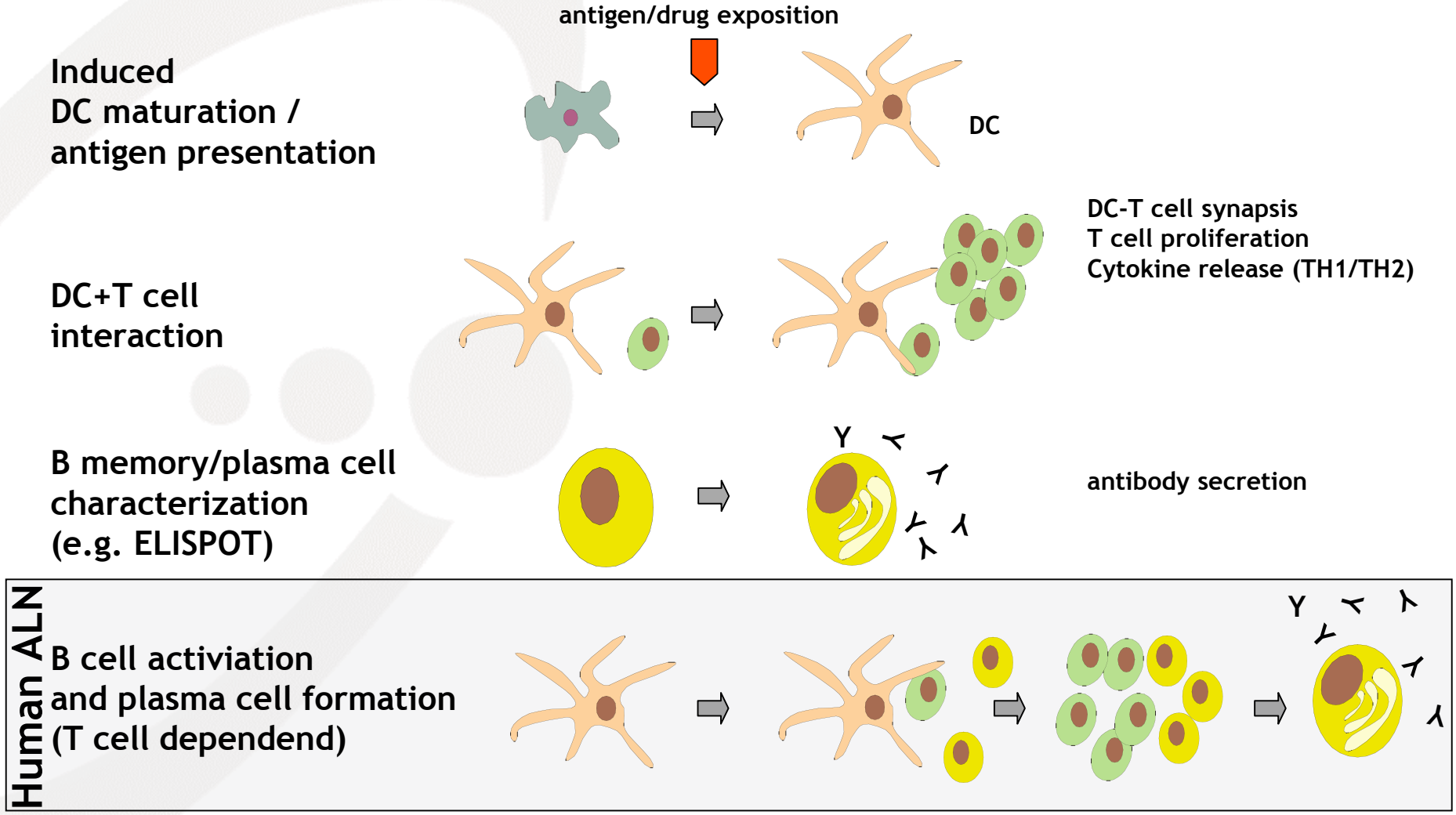
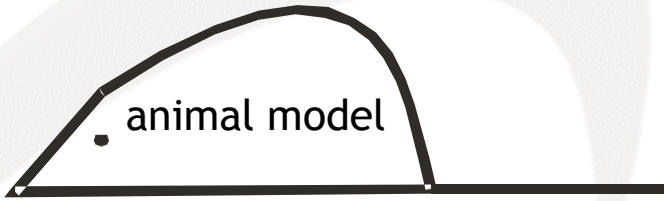
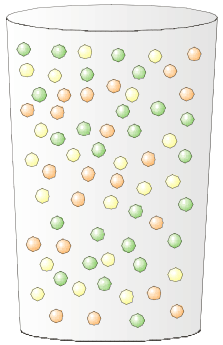


Limitations of *in vivo* and *in vitro* Testing





in vitro model

no human physiology

no tissue functionality
no long term culture

no human
tissue or organ functionality

The ideal artificial organ:
Human tissue or organ specificity and functionality

Underestimated Immunological Risks: The Patient Situation

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- Polymorphisms (HLA, other genotypical variations)

- Immune status and “immunological training” of donors, volunteers and patients

- Latent infection diseases

- “Innate environment” (danger signals)

- Patient disease status (immune system already affected?)

The Human Blood and Lymphatic System
In vitro: 3D matrix assisted and perfusion culture is crucial

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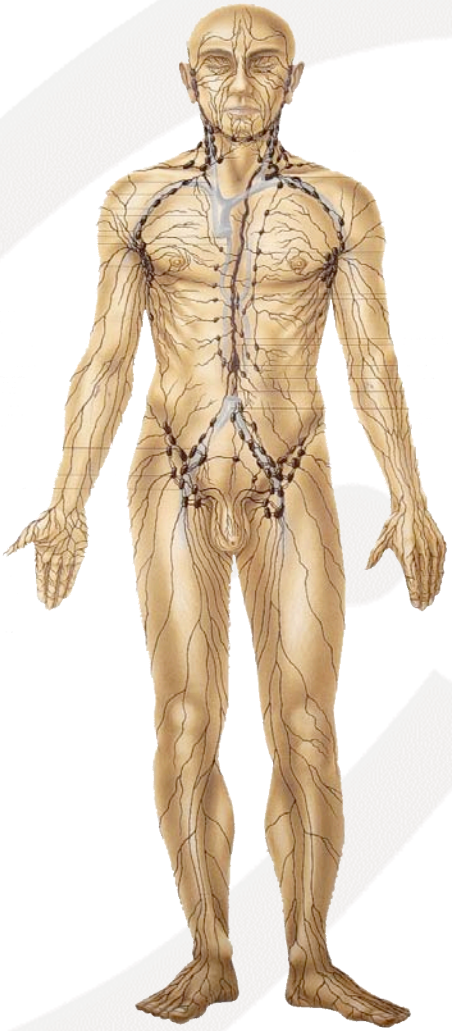
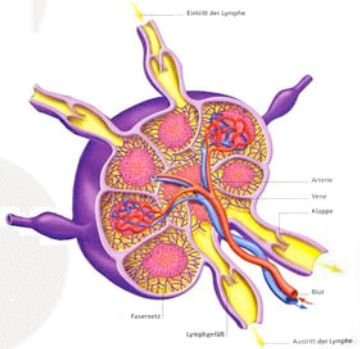
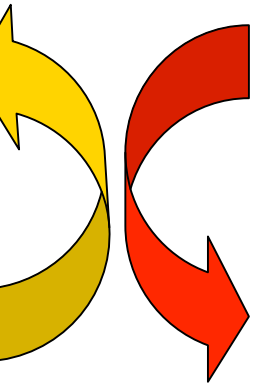
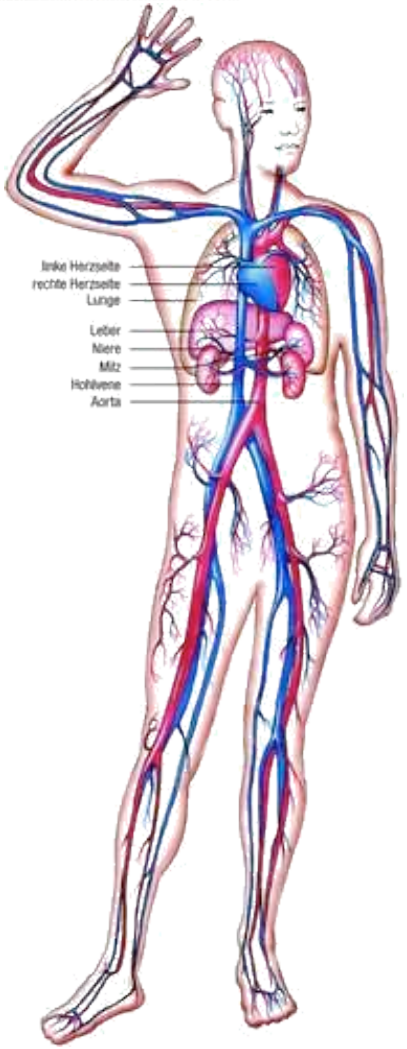


Lymphatic System

lymph nodes
as biological
"cross flow filters"







Blood System

Reference:
www.wissen.de/wde/generator/wissen/ressorts/kinder/forschen/special_koerperteile/index,page=10651
78,chunk=img_0.html © Wissen Media Verlag GmbH, Gütersloh

Migration and Homing of Lymphocytes in Lymphoid Tissue

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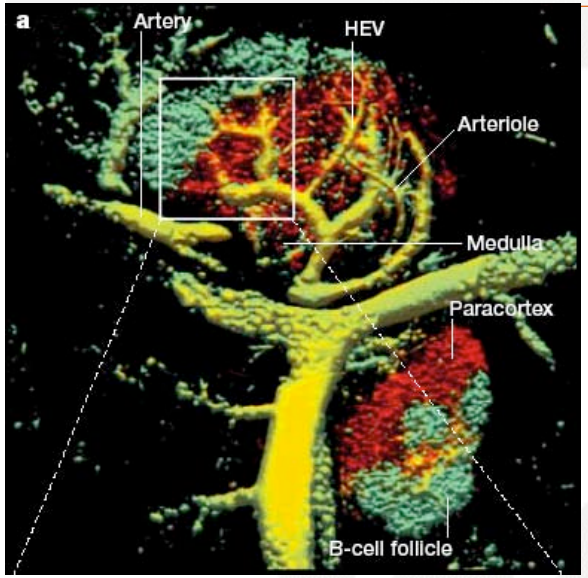
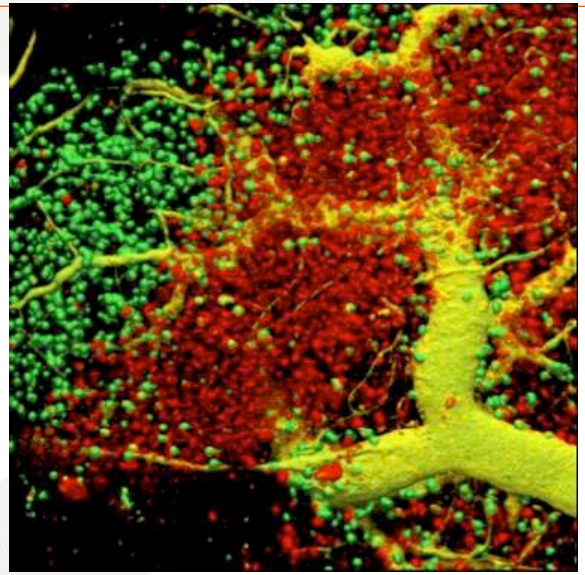




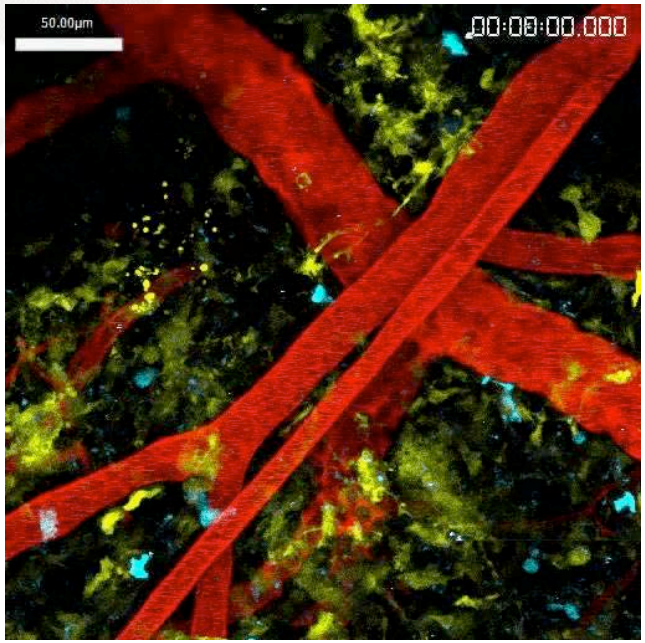
(*intra vital* microscopy @ mice)

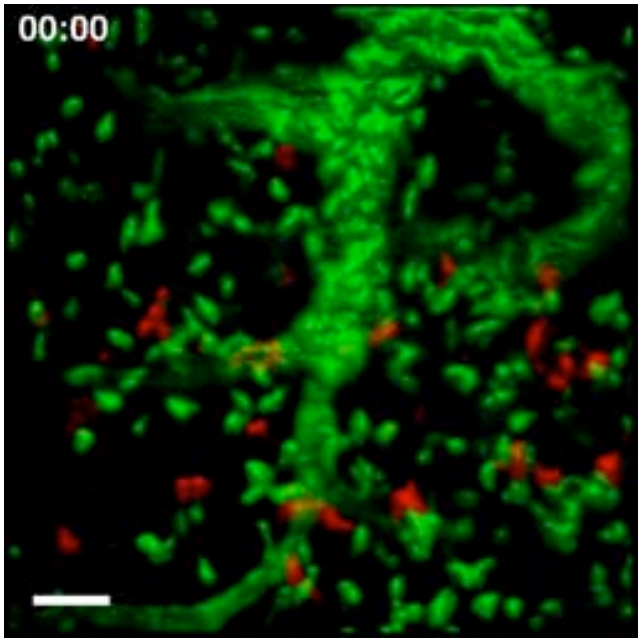
migration speed > 25 µm/min

Van Andrian and Mempel
(Immunol. 3(11), 2003)

Lindquist et. al.
(*Nature Immunol*. 5, 1243–1250, 2004)

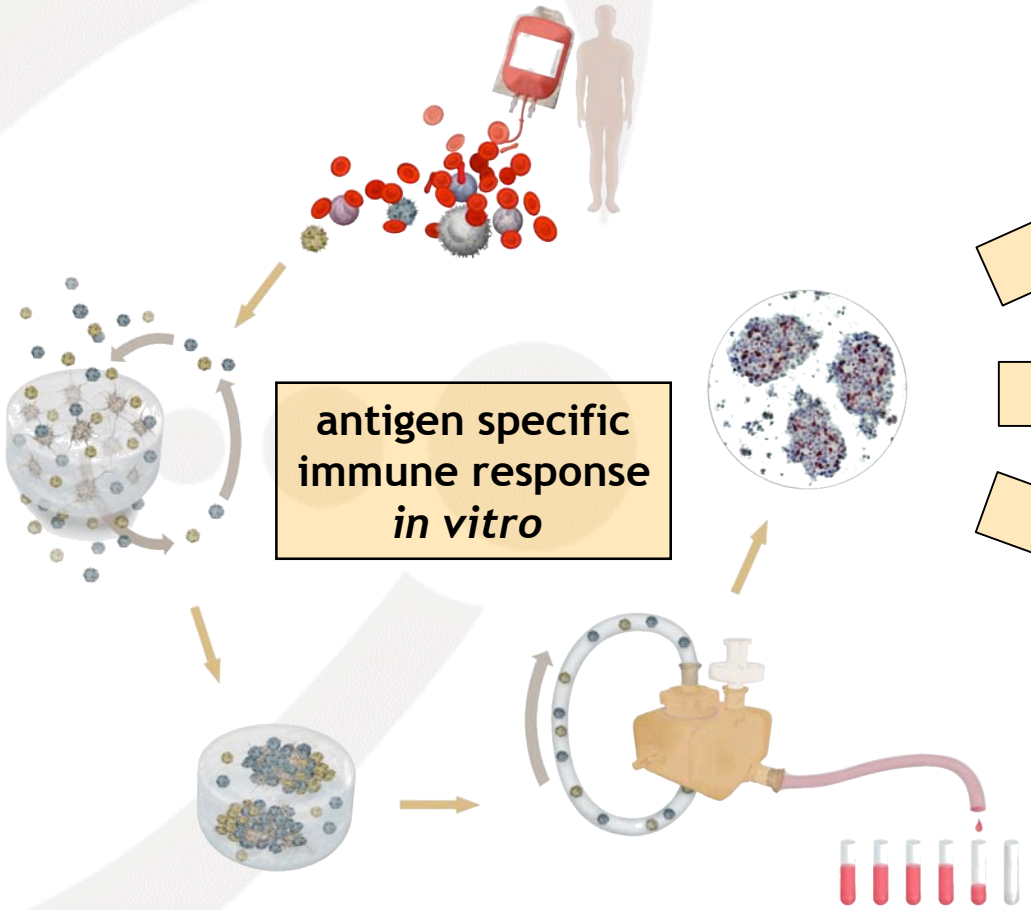
Stoll et. al.
(Science 7(296):1873-1876, 2002)





Human ALN
ecopa meeting, Nov 28, 2008

The Human Artificial Lymph Node Model (human ALN)



therapeutical use

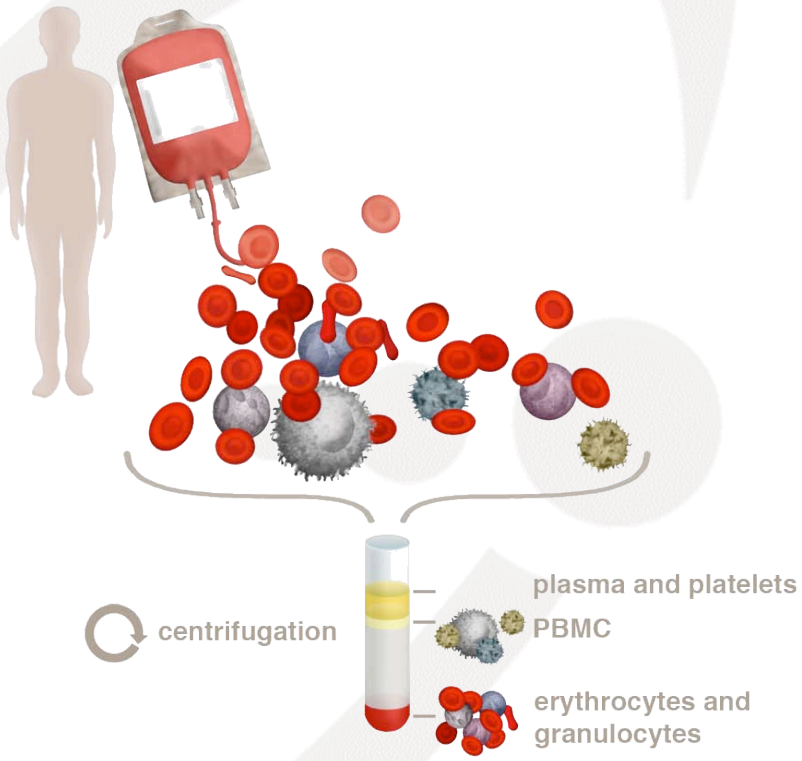
the B cell pathway **-**
generation of antibodies

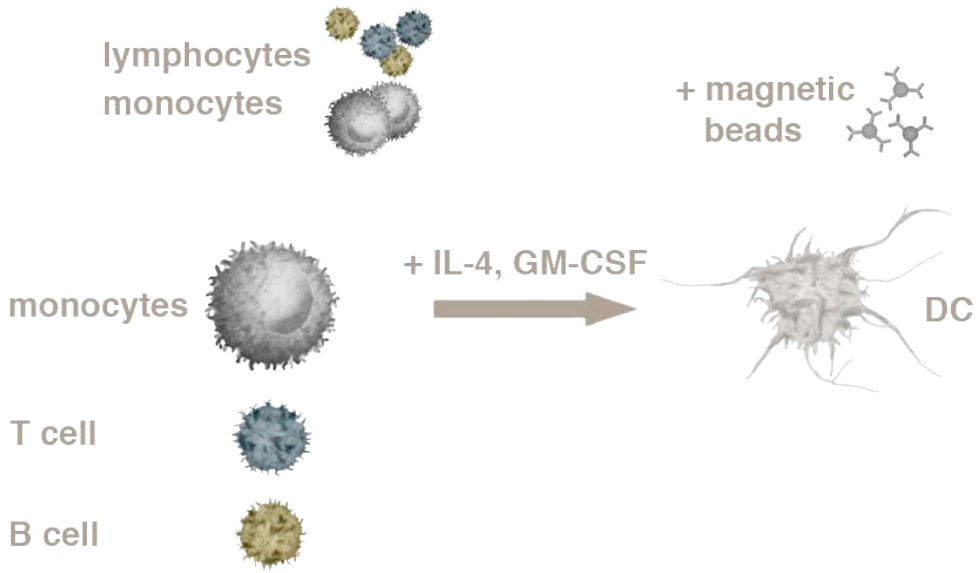
the *T cell pathway* -
generation of antigen specific T-cells

pharmacology
immunogenicity
immunotoxicity

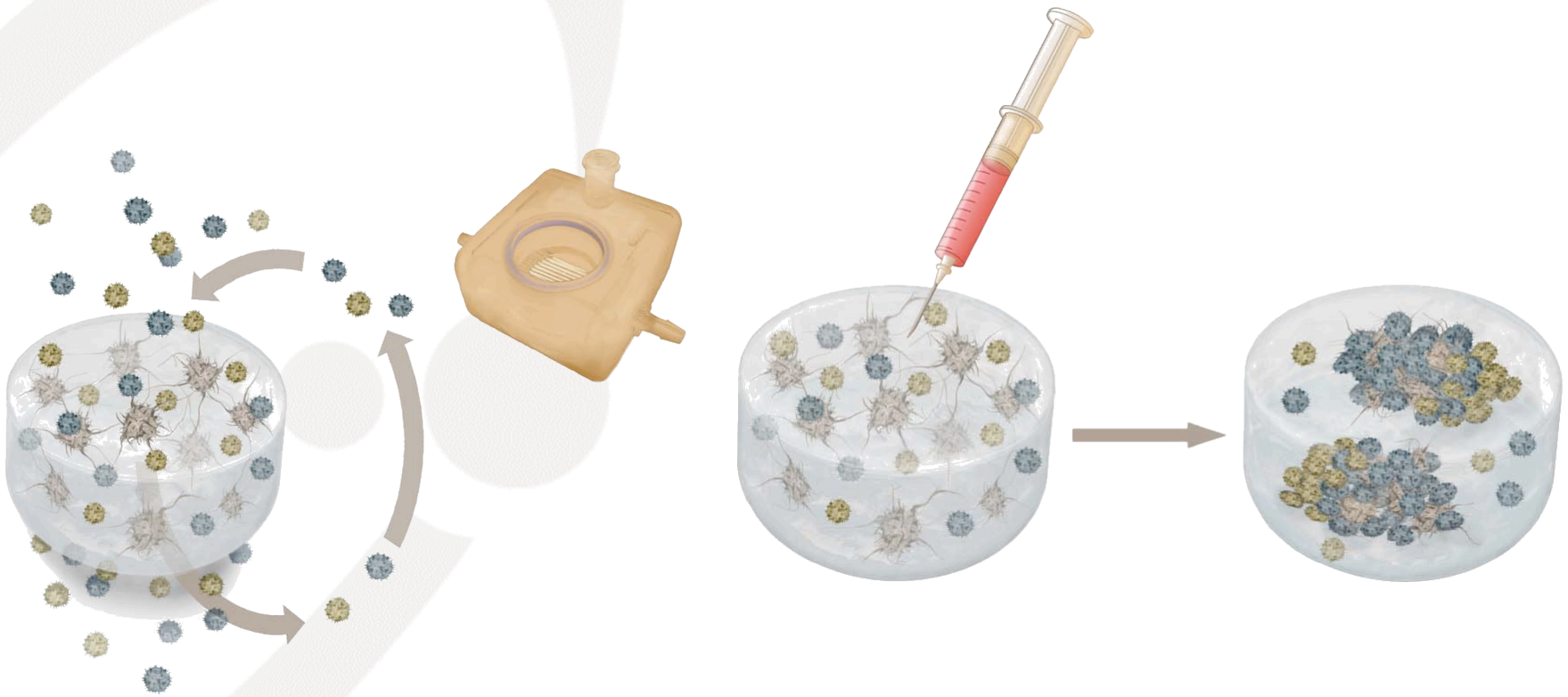
in vitro testing

Human ALN Model
Cell Preparation and DC Generation

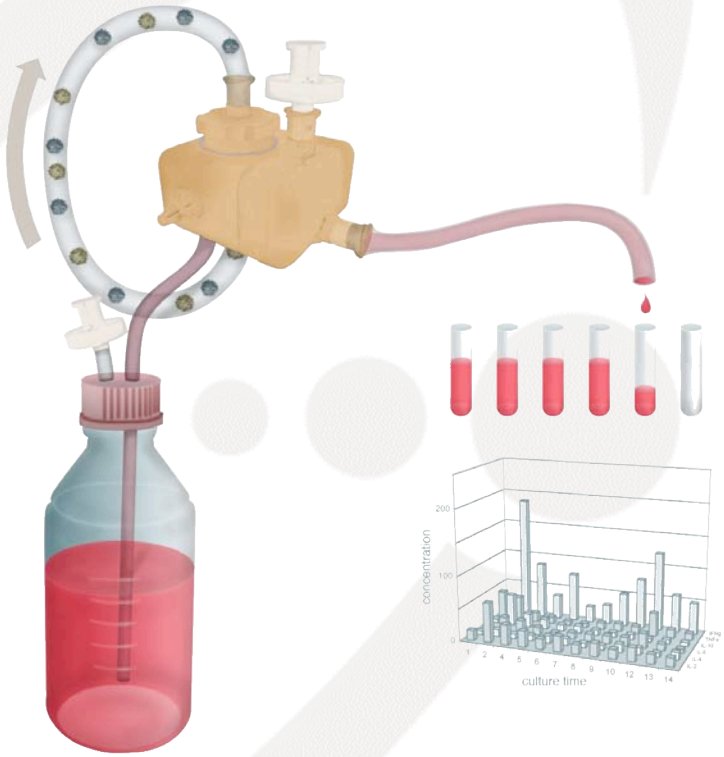


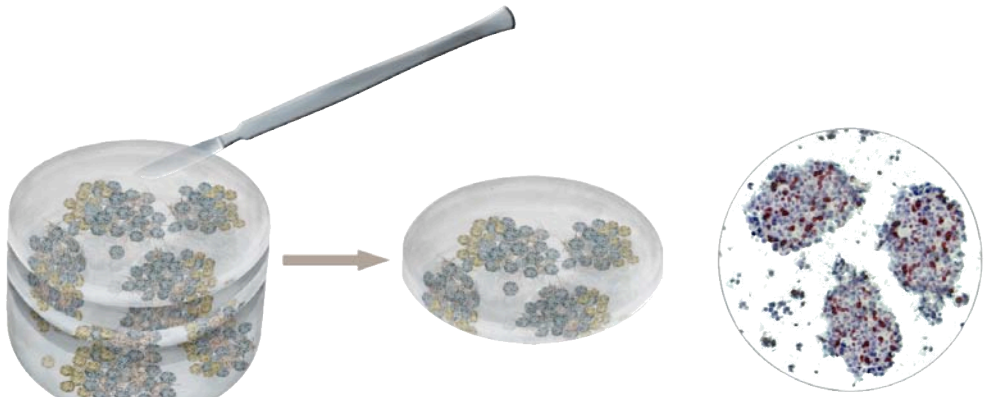


Human ALN Model
Bioreactor Inoculation and Organoid Formation

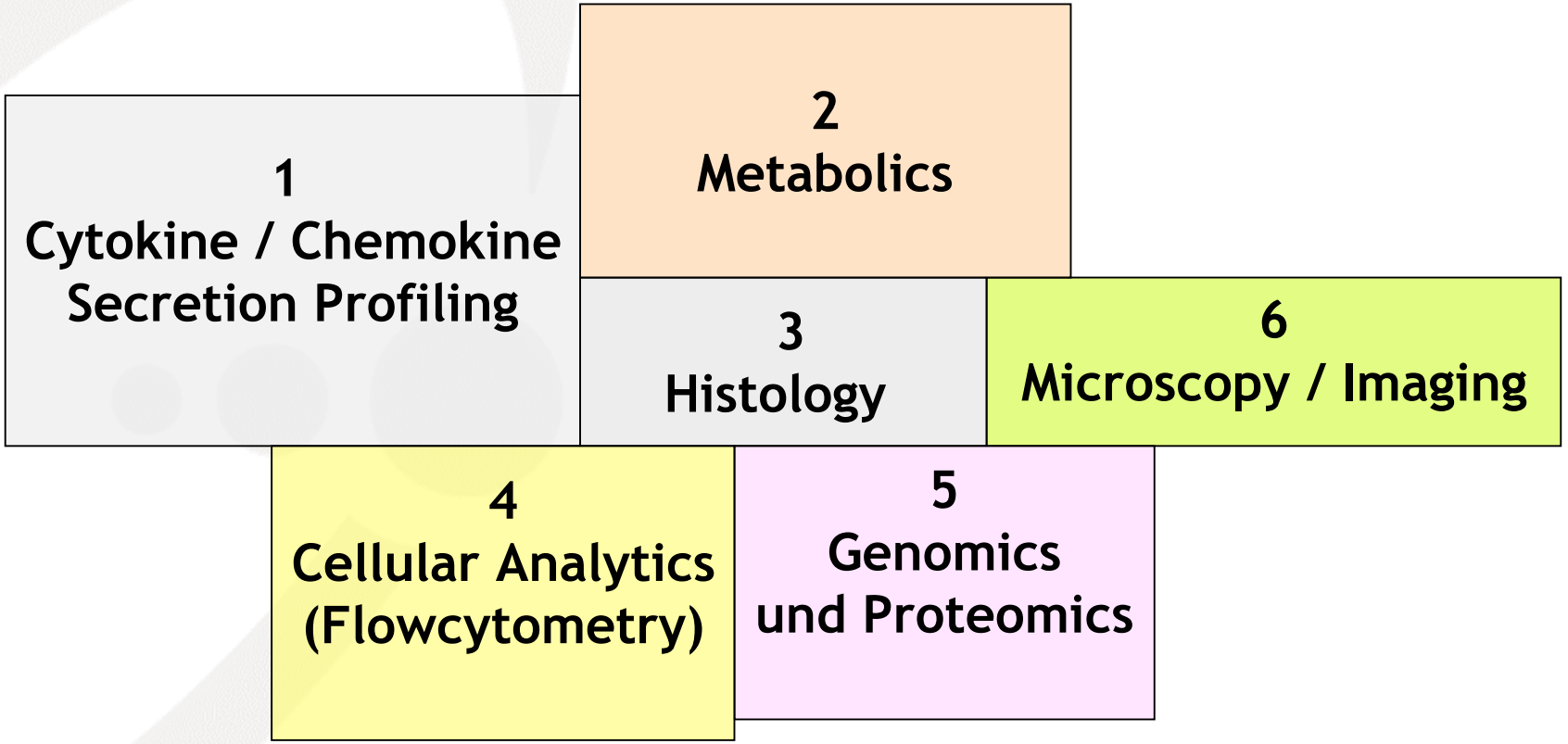


Human ALN Model
Online Monitoring and Histology





Human ALN Model
Read Out Parameters



Human ALN Model
Bioreactor Platforms (Medical Devices)

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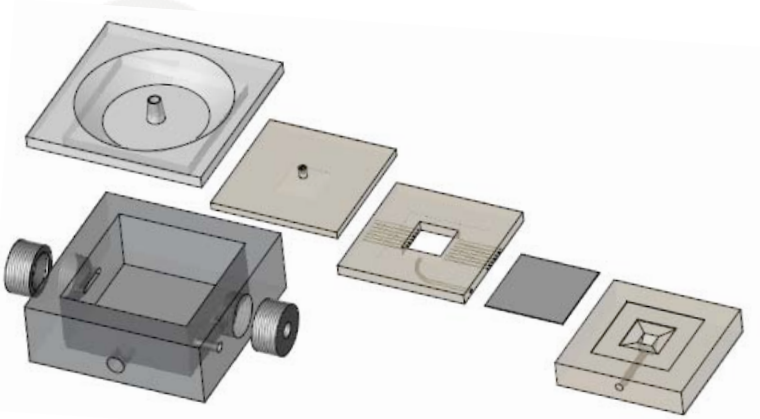
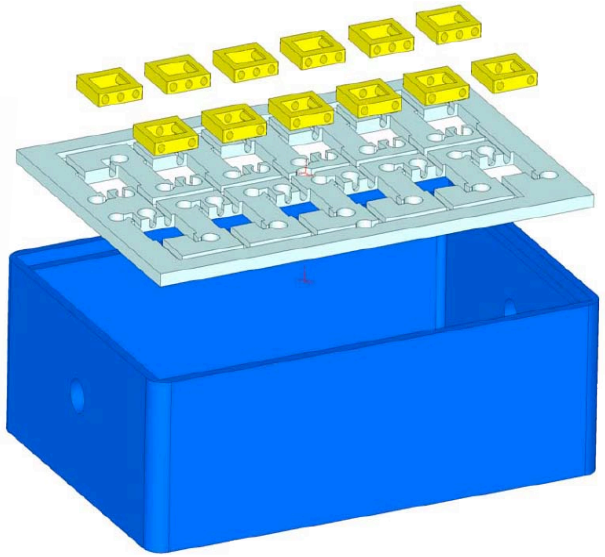
HIRIS 3 (industrial)
- large cell repertoire (10^8 DC/PBMC)
- cell perfusion

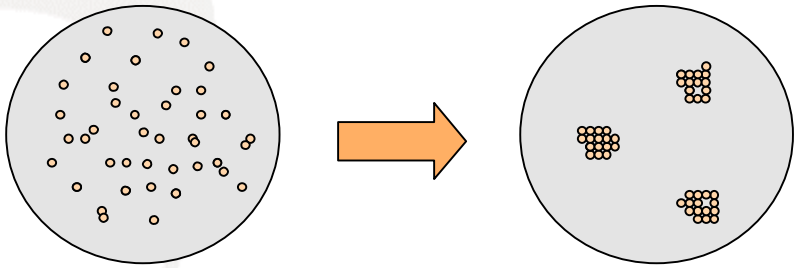
HIRIS 4 (prototyping)
- miniaturized
- reduced repertoire (10^7 to 10^8 DC/PBMC)
- cell perfusion
- multiparallel
- *in situ* imaging

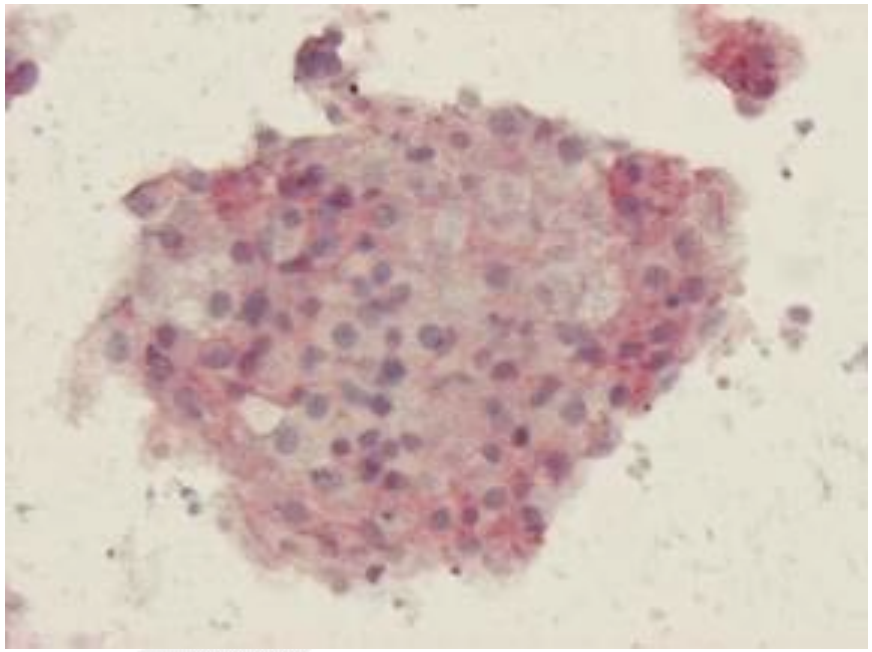


IG-Device (prototyping)
- miniaturized
- reduced repertoire (10^7 DC/PBMC)
- multiparallel
- *in situ* imaging

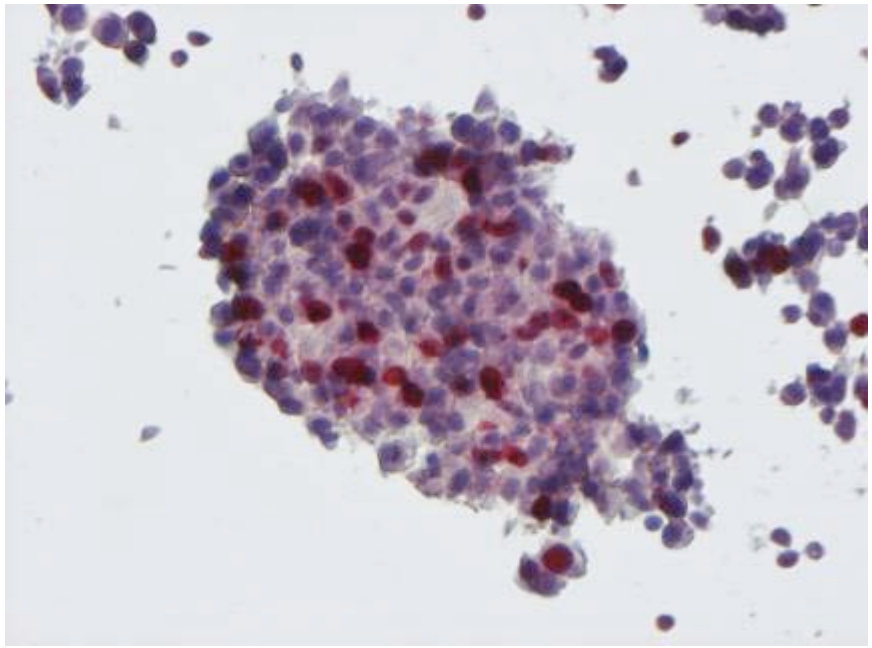


Human ALN Model
Clustering and Micro Organoid Formation





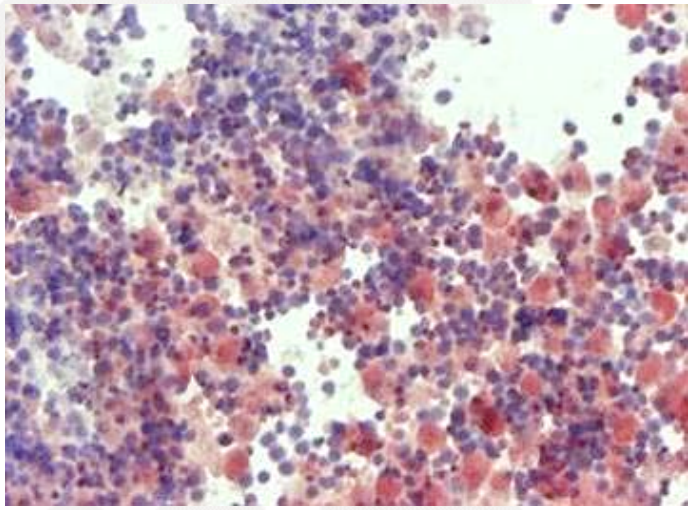
micro organoid formation in agarose matrix



cell proliferation in organoids (Ki67)

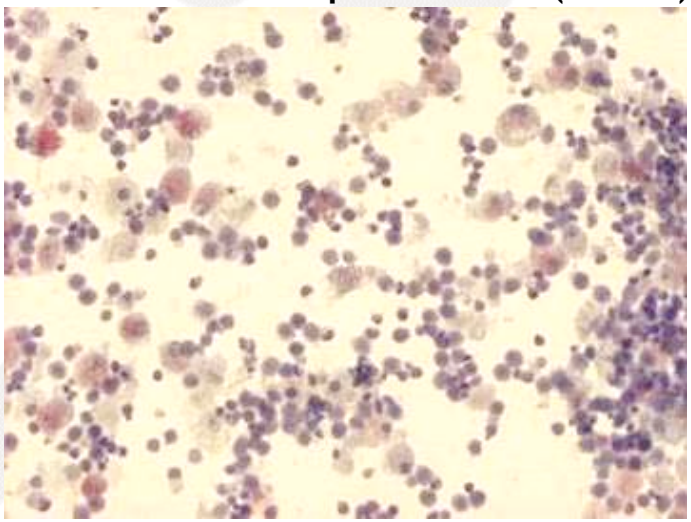
ABC+APhos+FastRed, hematoxylin

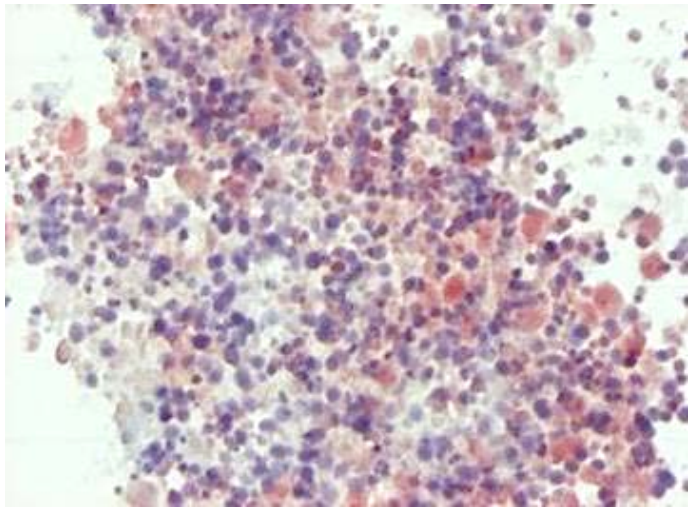
Plasma Cell Formation (CMV Exposition)



IgM

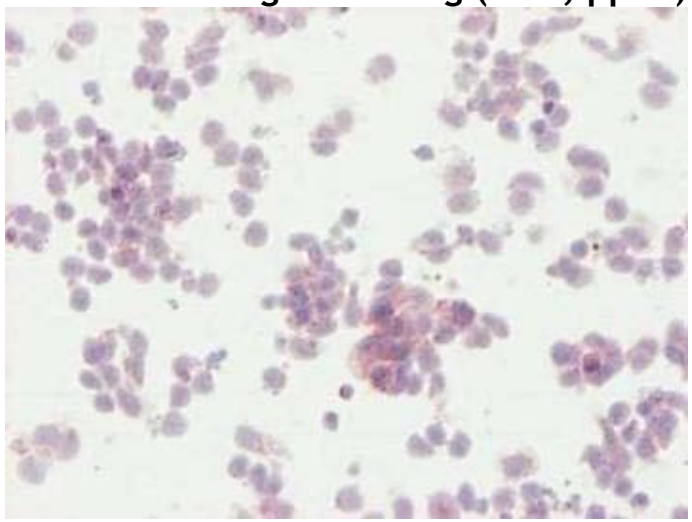
plasma cells (CD138)





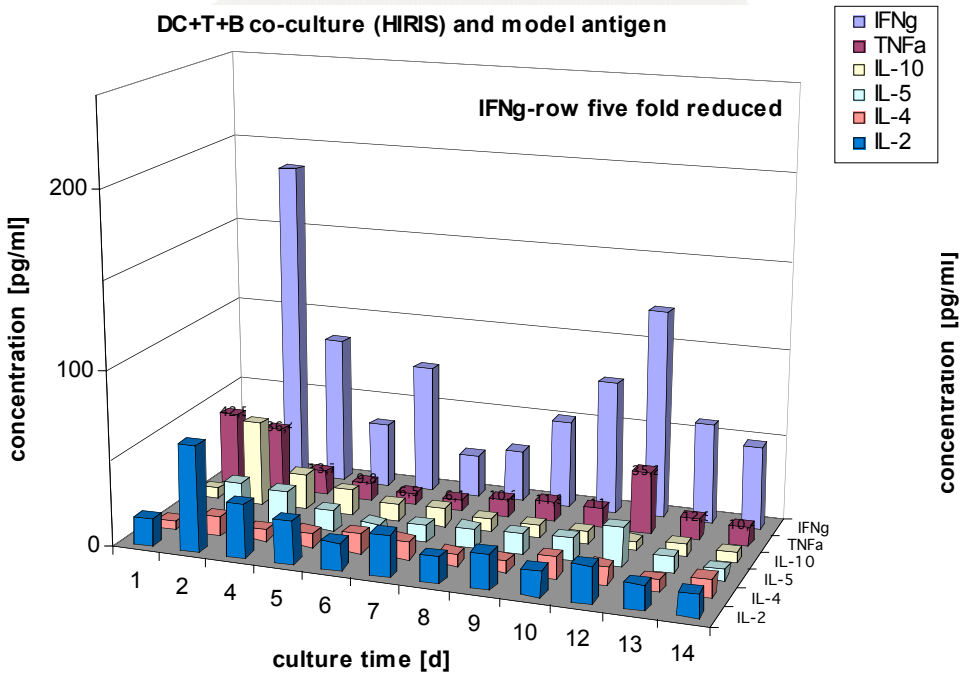
IgG

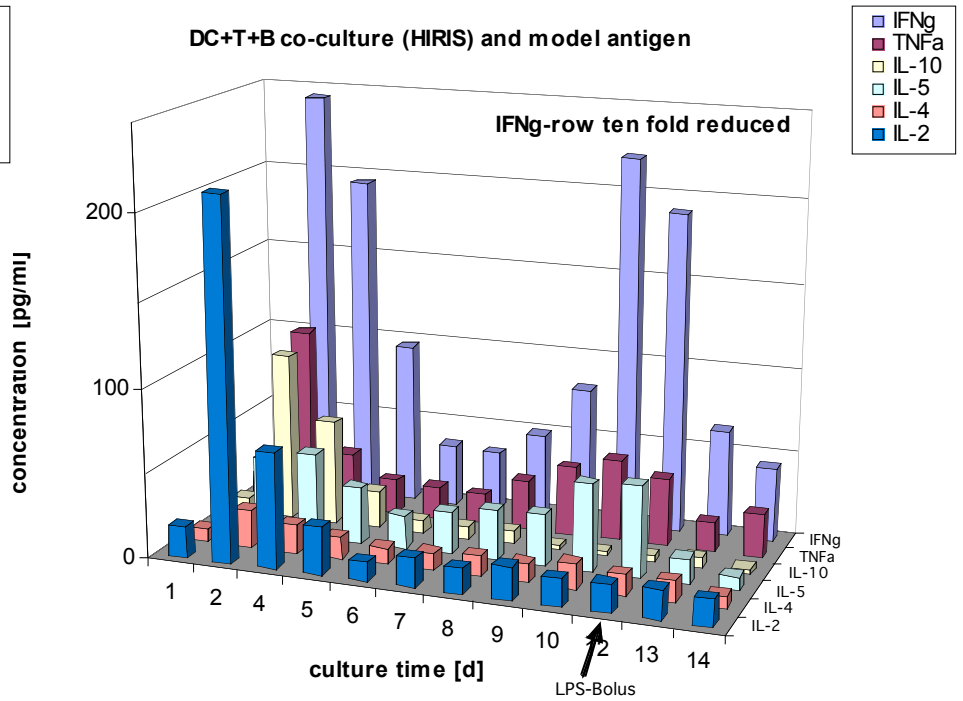
antigen binding (CMV, pp65)



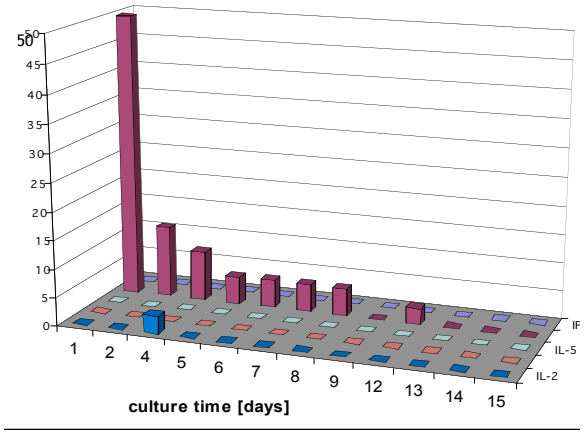
ABC+APhos+FastRed, hematoxylin, 40x

Immune Response against Viral Antigen (CMV)





negative control



CBA™-technology (BD), 6 plex
(lower detection limit 6-11 pg/mL)

"day 15"= media background

Human ALN
ecopa meeting, Nov 28, 2008

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Immune Response to Ovalbumin-Exposition

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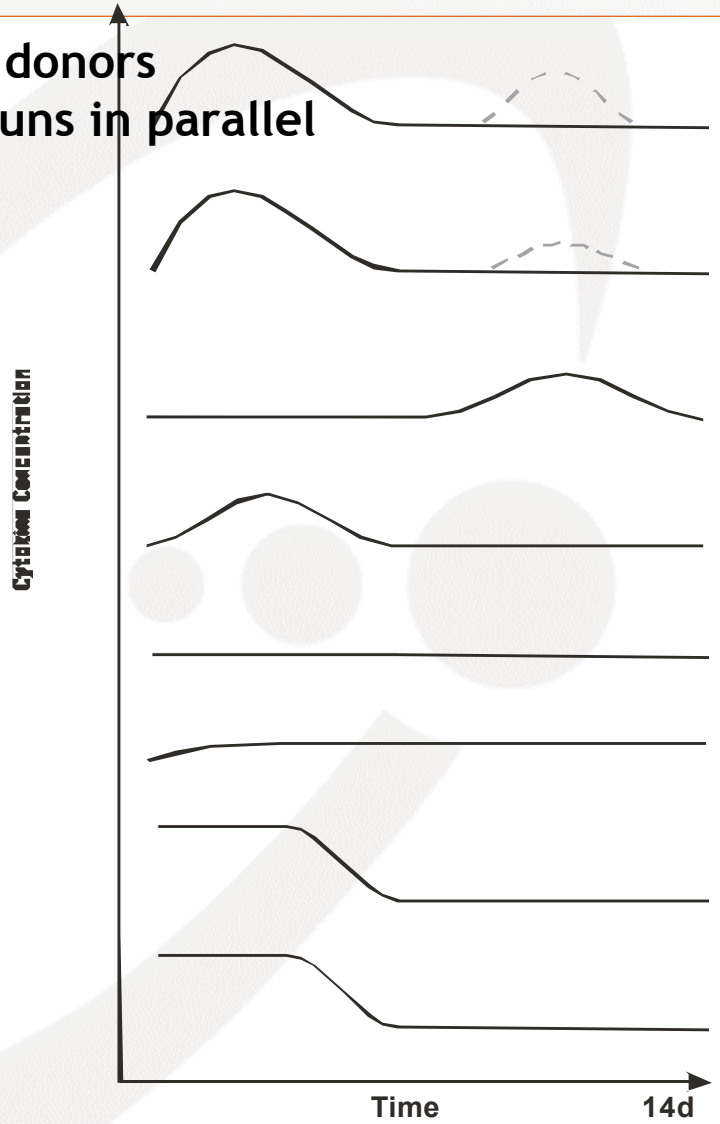
10 donors
4 runs in parallel



IFNγ	100-500	induced secretion per day [pg/ml]
TNFα	50-2000	
IL-5	20-100	
IL-2	20-100	
IL-10	-	
IL-4	100-1500	
IL-6	50-300	Bioplex™-technology 10 plex ultrasensitive (lower detection limit 0.8 1.5 pg/mL)
IL-1b	~10	background: below lower detection limit (exept IFNγ 1-10 pg/mL)

Human ALN
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ProBioGen AG

Immune Response to Albumins using the Resting Lymph Mode Model

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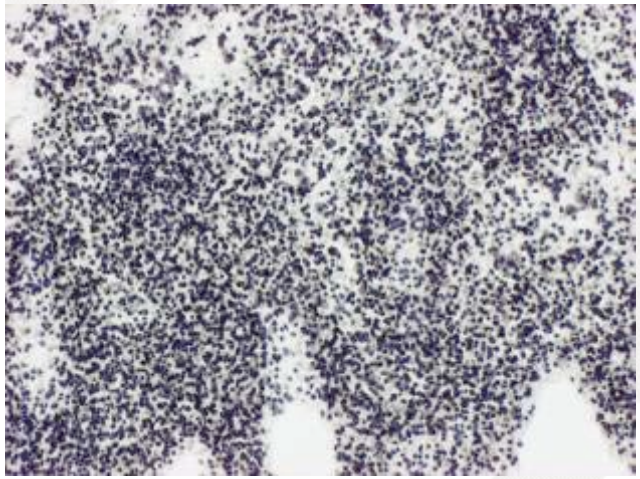
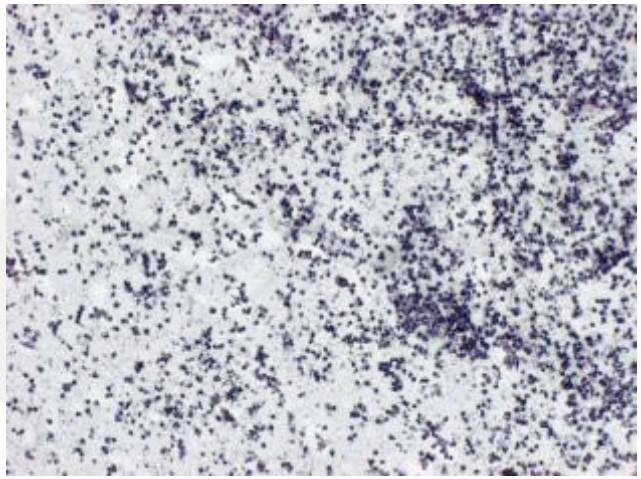
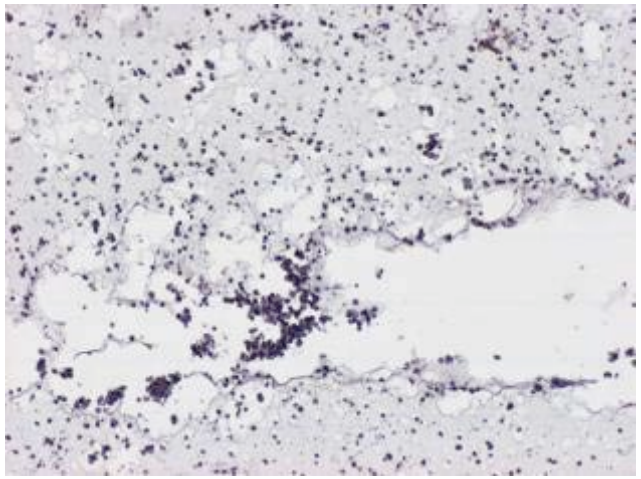
-induced cell proliferation-



Ovalbumin



bovine Albumin



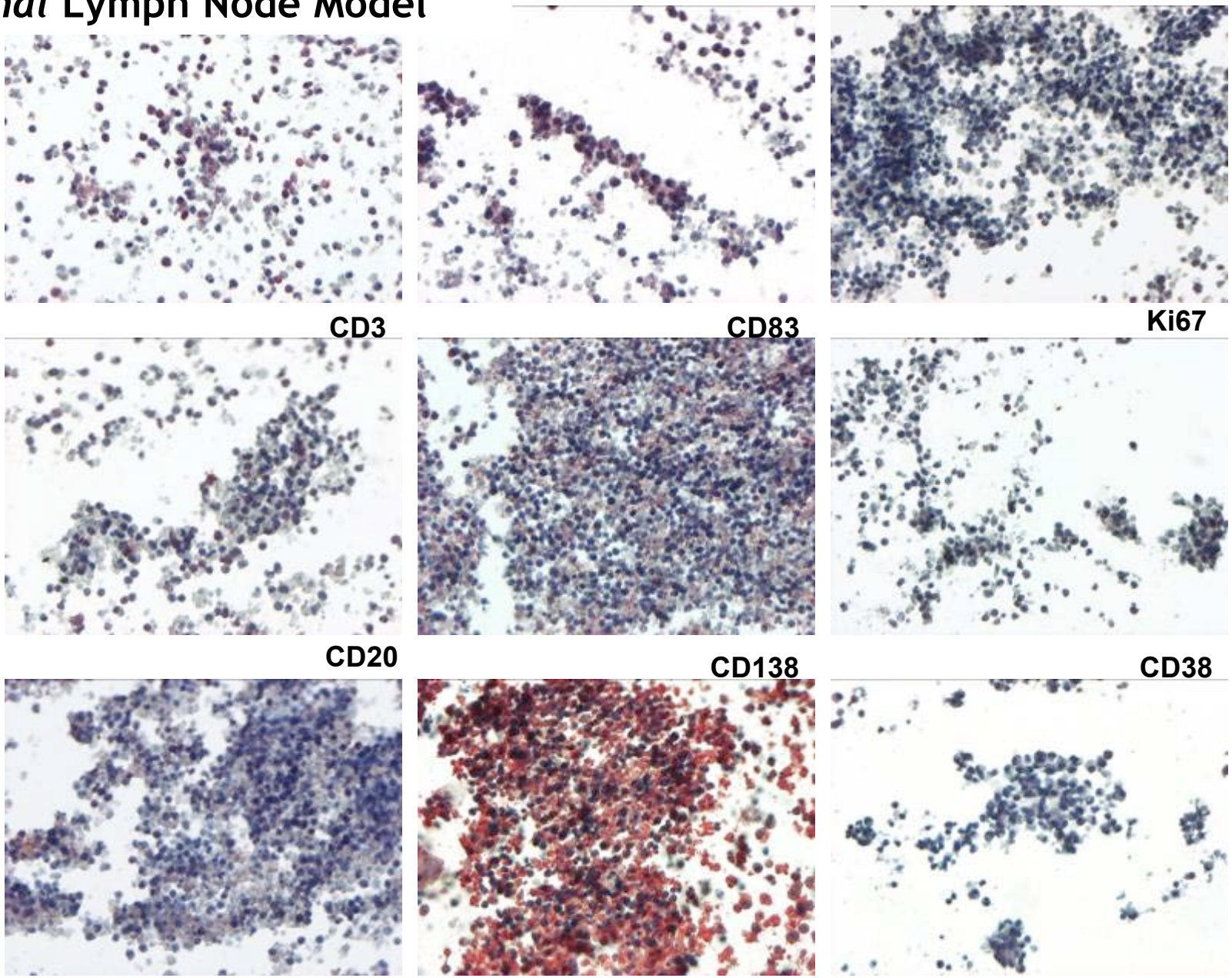
human Albumin

hematoxylin, 10x

Conventional Lymph Node Model

(Ovalbumin)

#108



ABC+APhos+FastRed, hematoxylin, 20x

Conclusions I

- ProBioGen is developing the model of a **human Artificial Lymph Node**
- The **human ALN**, emulates both, **humoral** and **cellular** immune responses *in vitro*
- The model may be used for **predictive testing** of immunofunction and immunotoxicity
- Current testing programmes for pharmaceutical customers:
  Interferons (IFN α/β)
  Superagonists (CD3 / CD28 MAbs)
  Vaccines and **adjuvant therapy**

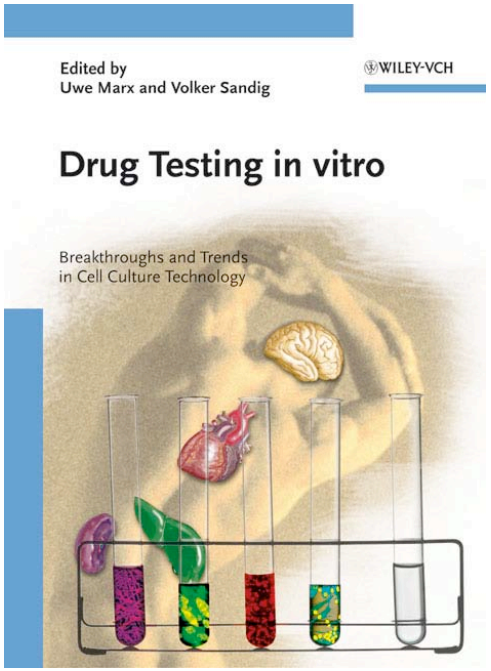
Conclusions II

- Current testing programmes for **cosmetical industry**:
  cell based assays (DC and DC+T cell activation assays)

- ProBioGen plans to extend **collaborations** and **testing services** using the **human ALN model** from current pharmaceutical to cosmetical and chemical applications

- ProBioGen is actively **looking for collaborations** to apply feasibility studies on pharmaceutical relevant drug candidates and compounds

Acknowledgements



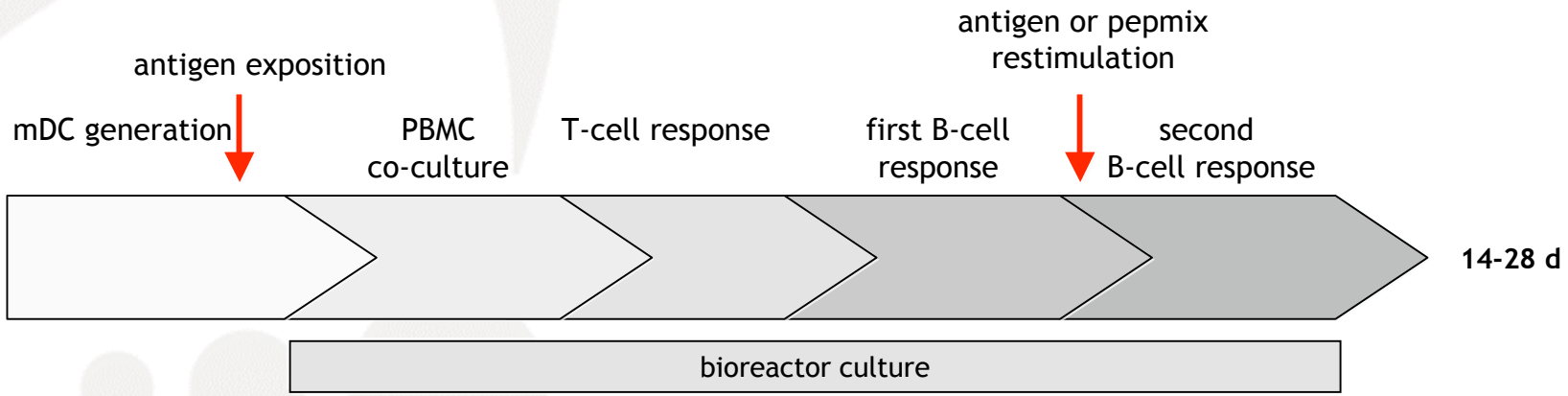
Hans-Dieter Volk (Charité, Berlin)
Claudia Berek (DRFZ, Berlin)

Uwe Marx, CSO ProBioGen AG
and the Team of Cell And Tissue Services (ProBioGen AG)

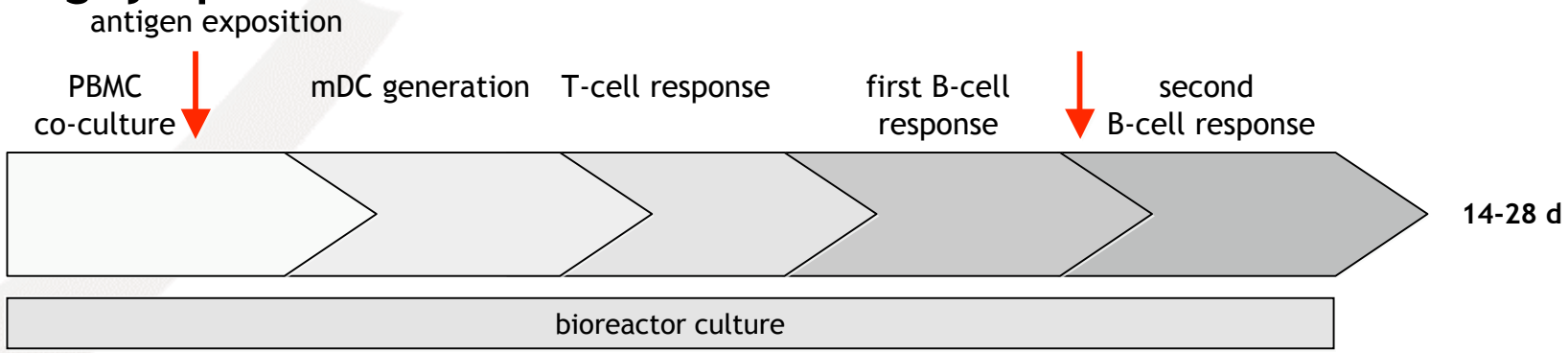
Thank you.

Process of Cultivation and Exposition

conventional lymph node model (preformed mDC)



resting lymph node model



Test methods *in vitro*
Graded Levels of Validation for Assay Applications

- drug screening
- lead optimisation
- manufacturing (process development)
- formulation, packaging, storage
- batch release of tox material,
  clinical material and final product
- clinical monitoring, patient samples
  (immunotoxicological monitoring)