In vitro systems for assessing vaccine quality and elucidating vaccine-induced immune mechanisms

Aurora Signorazzi[1], Marilena Etna[2], Gabriela Tapia Calle[1], Shuran Gong[1], Eliana Coccia[2] and Anke Huckriede[1]

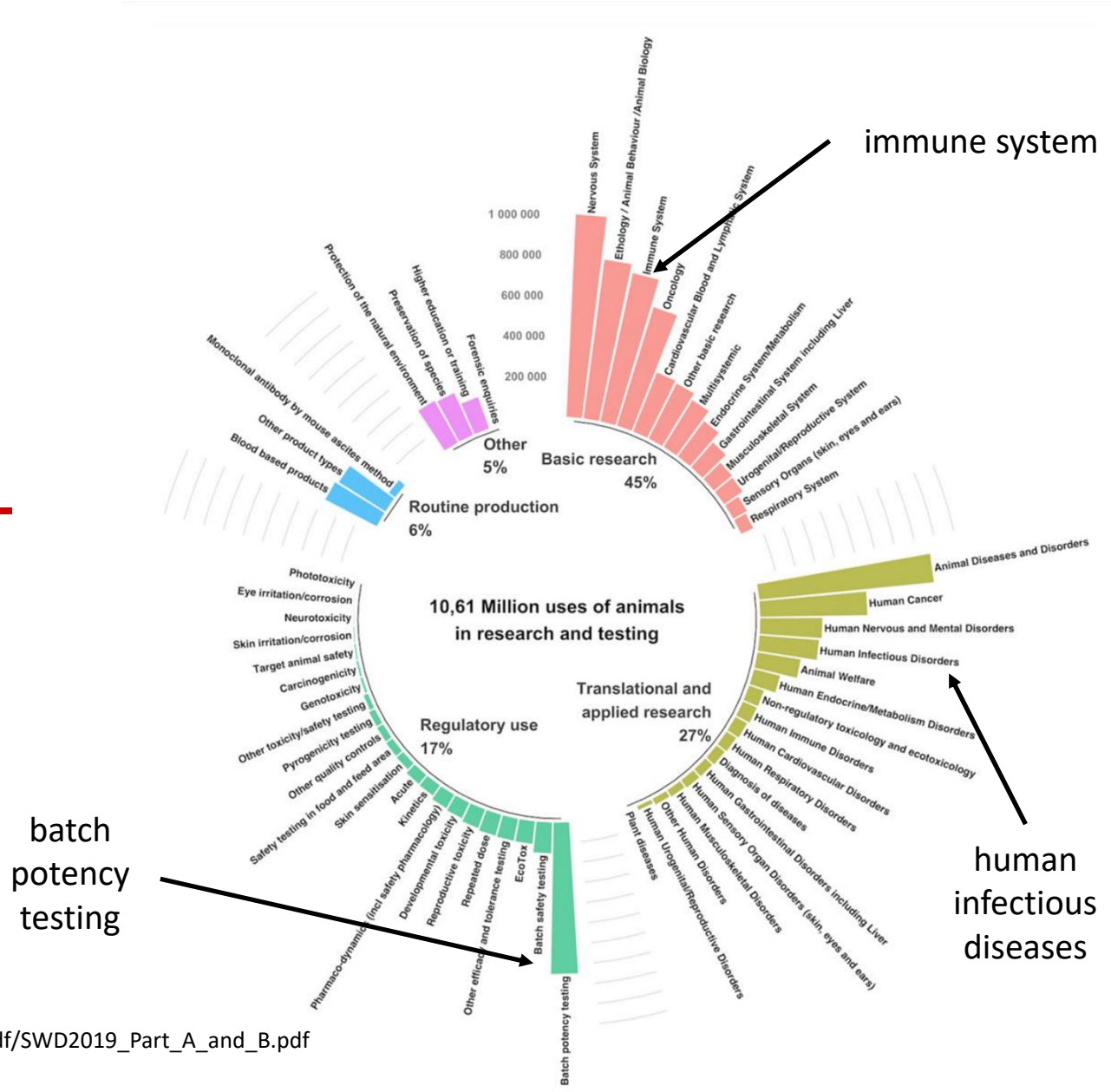
[1] Department of Medical Microbiology & Infection Prevention, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands

[2] Department of Infectious Diseases, Istituto Superiore di Sanitá, Rome, Italy

university of groningen

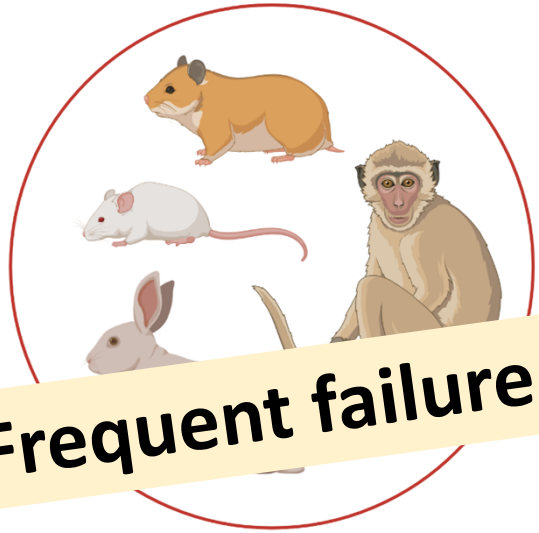
umcg

Use of animals in research and production (EU + Norway 2019)



https://ec.europa.eu/environment/chemicals/lab_animals/pdf/SWD2019_Part_A_and_B.pdf

Animals are not small humans



Differences in cytokines and immune receptors

Very different life span

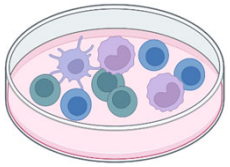
neterogeneity

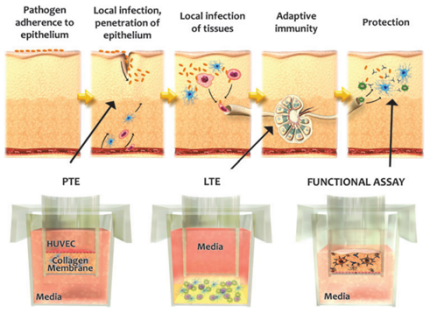
Different immune history

Frequent failure of vaccine candidates in clinical trial

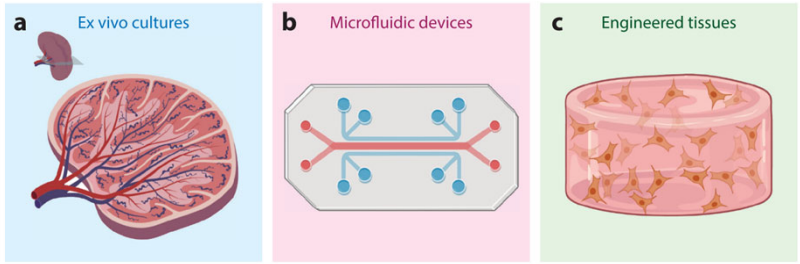
Modeling the human immune system *in vitro*

- Immune response induction
  - Blood cell-based models
  - Artificial lymph nodes
  - MIMIC®
- Immune response effects
  - Organ slices
  - Organ-on-a-chip
  - Organoids





Drake III et al Disruptive Sci & Techn 2012

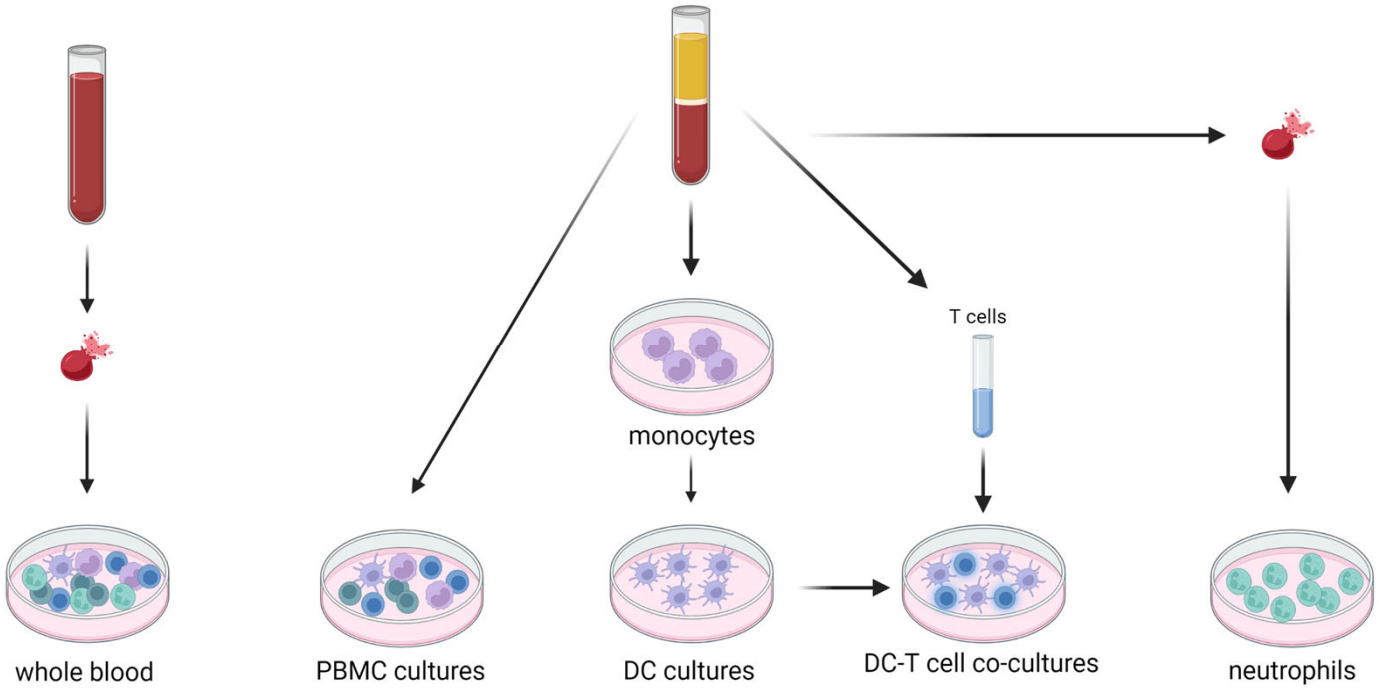


Hammel et al Ann Rev Biomed Eng 2021

university of groningen

umcg

Simple blood cell-based systems

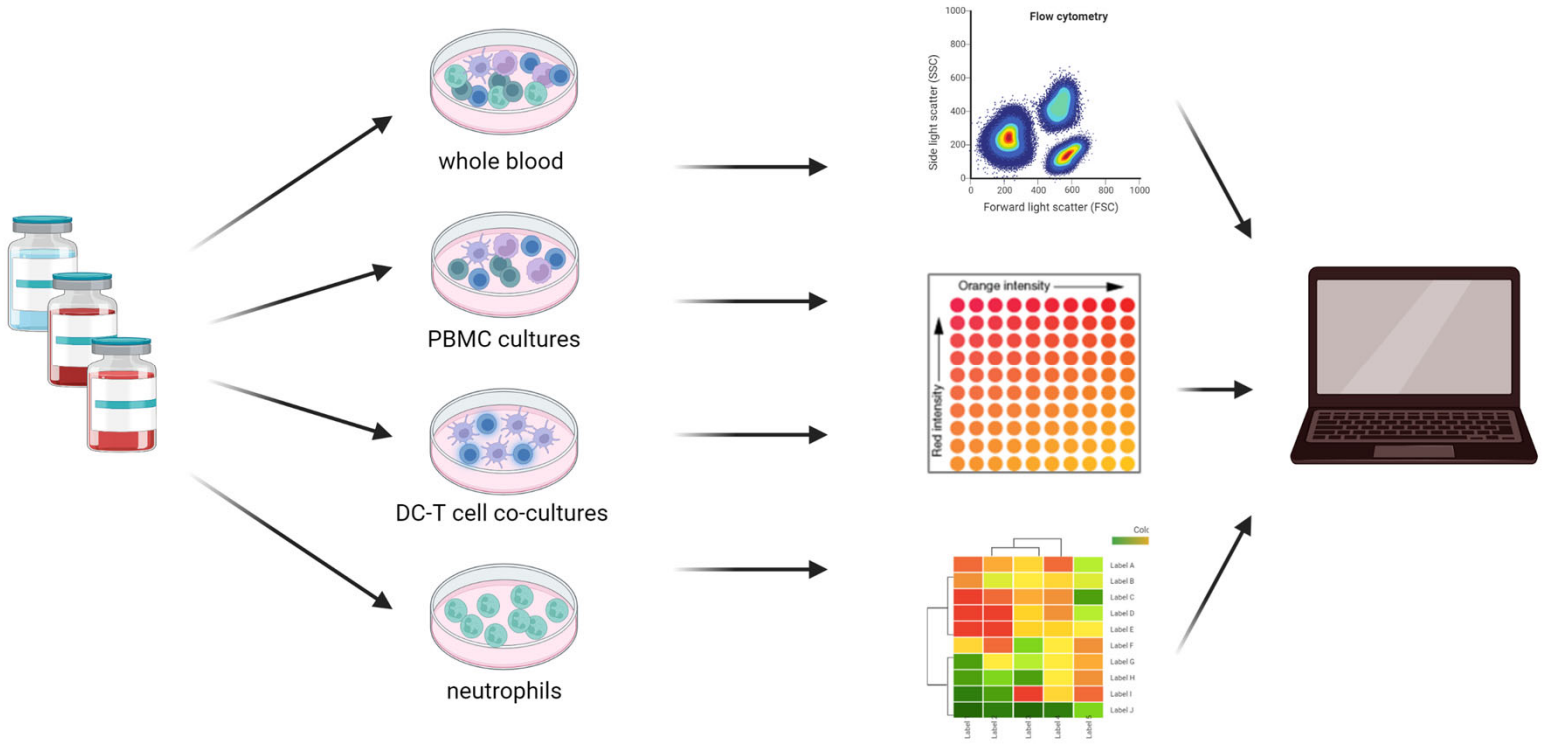


university of groningen

Created with BioRender.com

umcg

Experimental setup



university of groningen

Created with BioRender.com

umcg

Example 1:
Use of PBMCs for vaccine comparison

university of groningen

umcg

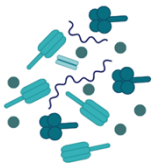
The model: Different types of influenza vaccines

- High immunogenicity in naïve individuals

- Th1-like response
- High IgG2a
- High levels of IFNγ-producing T cells

whole inactivated virus

split

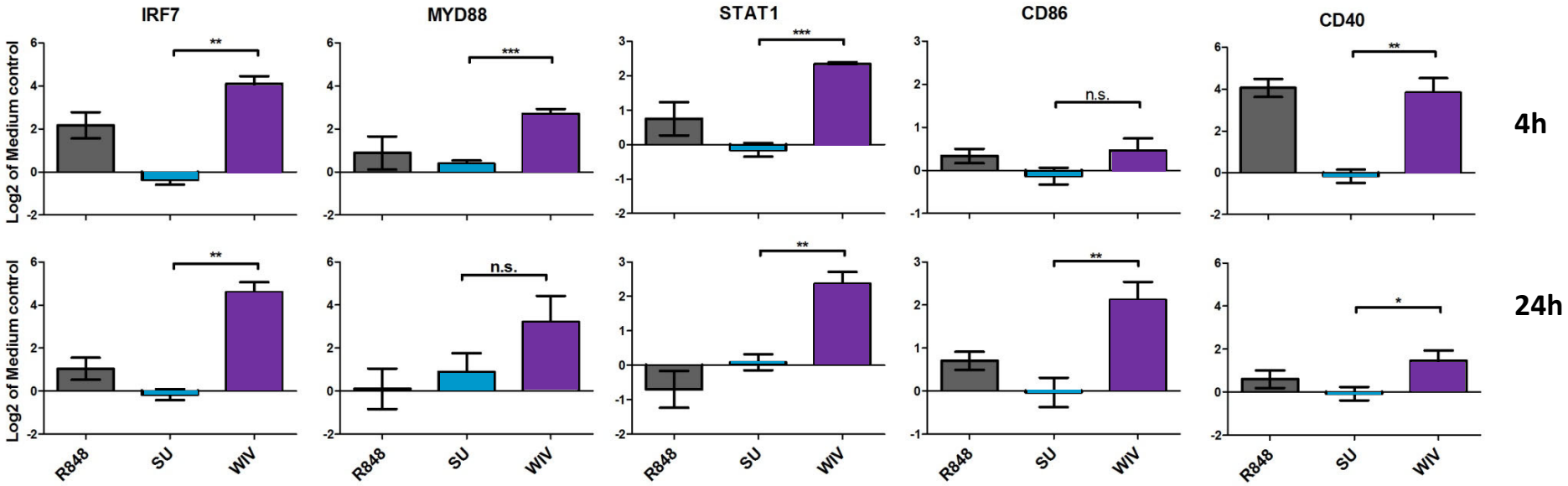


subunit

- ‘Low’ immunogenicity in naïve individuals

- Th2-like response
- High IgG1
- High levels of IL4-producing T cells

WIV but not SU vaccine activates distinct immune related gene expression pathways in DCs



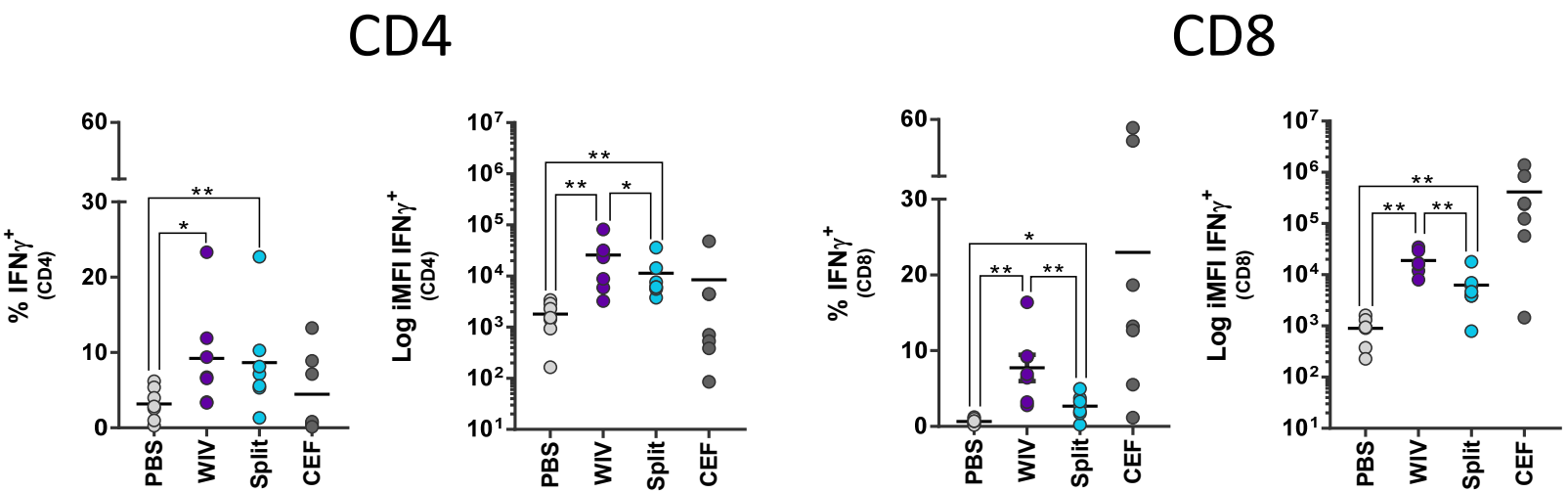
Note: response to R848 and WIV similar but not identical!

Tapia-Calle et al Vaccines 2017

university of groningen

umcg

WIV is superior to split vaccine in inducing IFNγ production in CD4 and CD8 T cells

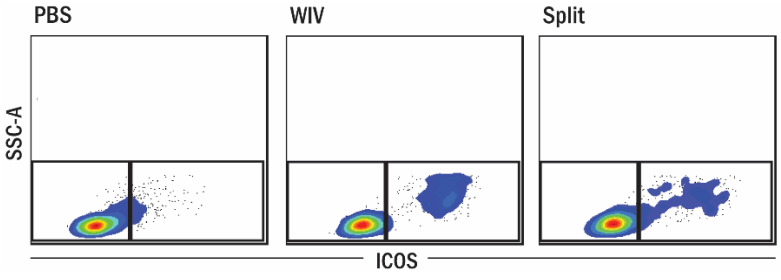


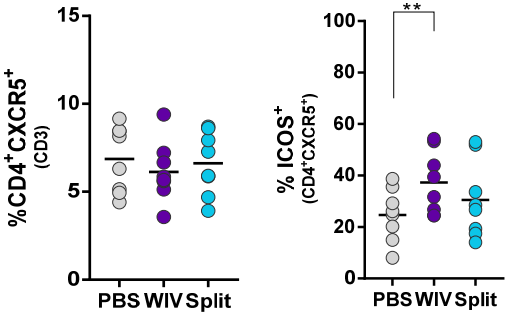
Tapia-Calle et al Vaccines 2017

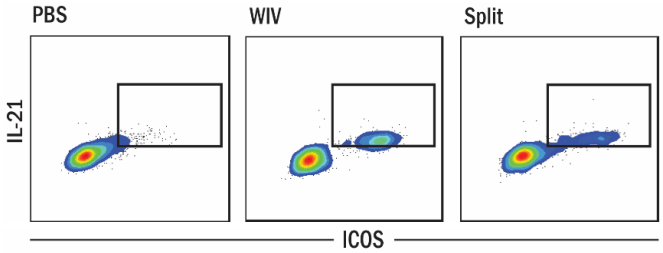
university of groningen

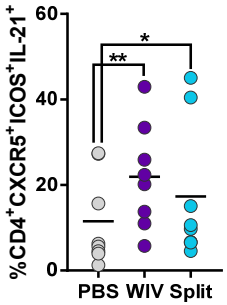
umcg

WIV and split vaccine stimulate T_{FH} cells which are associated with antibody responses









university of groningen

Tapia-Calle et al., Vaccines 2019

umcg

Example 2:
Use of PBMCs in vaccine batch quality control for Tick Borne Encephalitis (TBE) vaccine

university of groningen

vac2vac

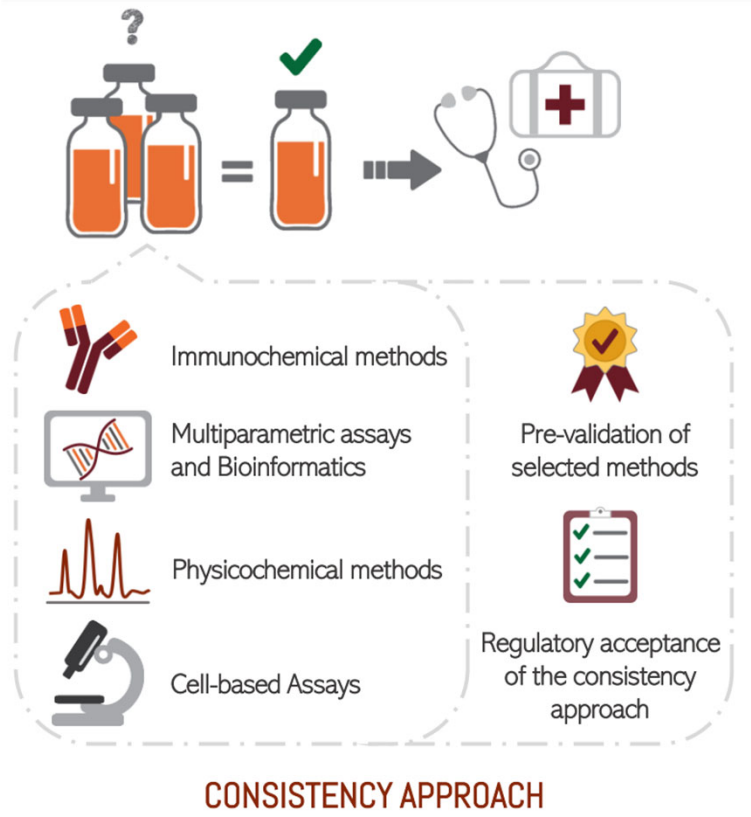
umcg

Current approach in TBE vaccine quality control

- Vaccine: Formaldehyde-inactivated virus adjuvanted with Alum
- Vaccine batch assessment:
  - Physico-chemical characterization
  - Mandatory animal challenge experiments to prove and measure vaccine potency
- Problems
  - Large number of animals required
  - High level of discomfort for animals
  - Large variation in outcome of challenge experiments (accepted confidence interval 30-300%)

Alternative approach for TBE vaccine quality control

- Batch-to-batch consistency approach

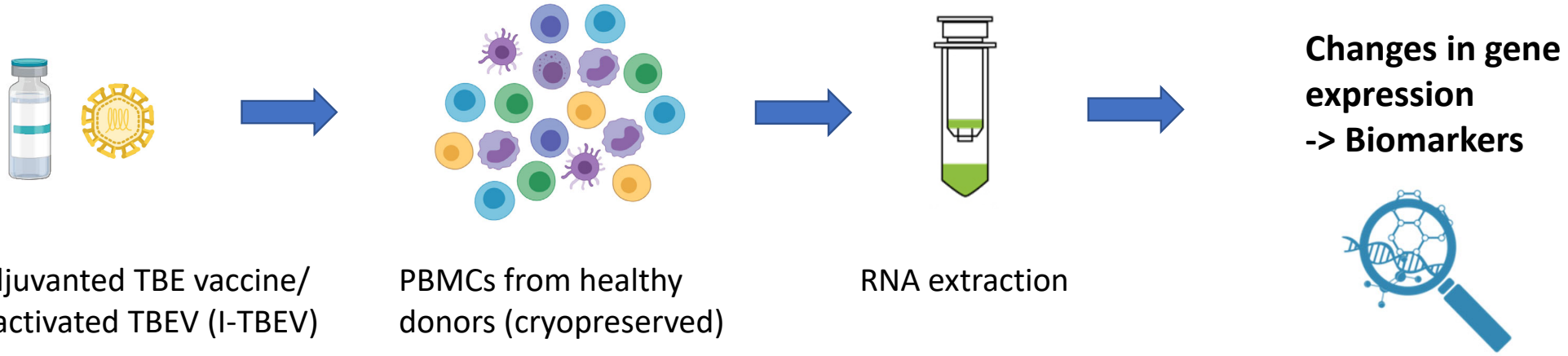


university of groningen

umcg

Quality assessment of tick-borne encephalitis(TBE) vaccine batches

Aim: Identify biomarkers for quality/potency of vaccine



Adjuvanted TBE vaccine/ inactivated TBEV (I-TBEV)

PBMCs from healthy donors (cryopreserved)

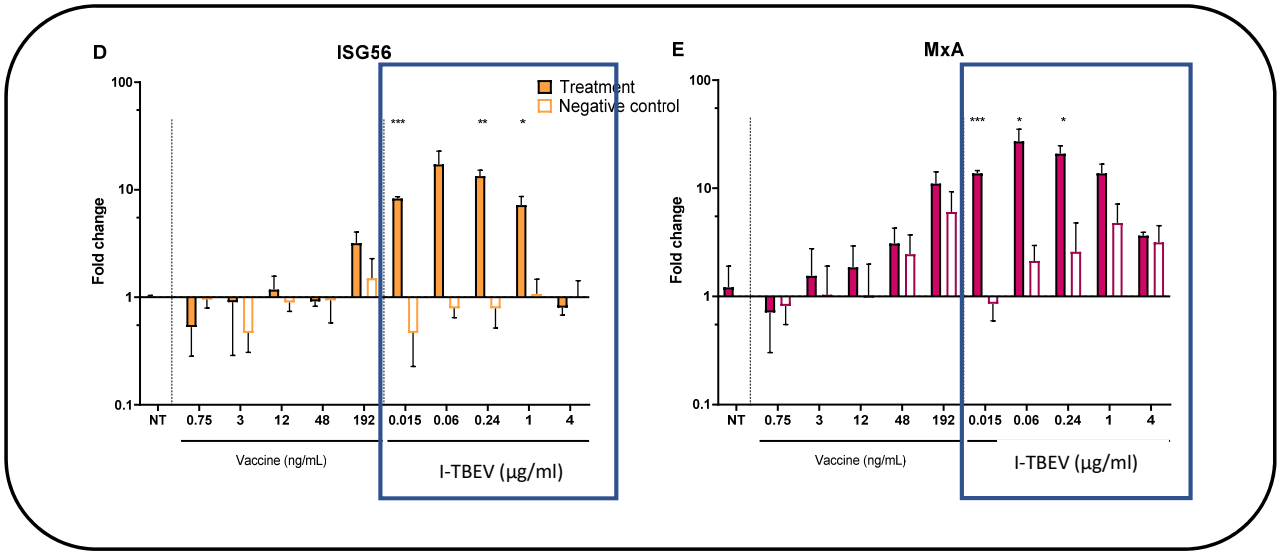
RNA extraction

university of groningen

Collaboration with Eliana Coccia & Marilena Etna, Istituto Superiore di Sanità, Rome, Italy

umcg

Human PBMCs respond to inactivated-TBEV (I-TBEV) by upregulation of innate immune responses



university of groningen

Signorazzi, Etna, Coccia & Huckriede ALTEX 2021

umcg

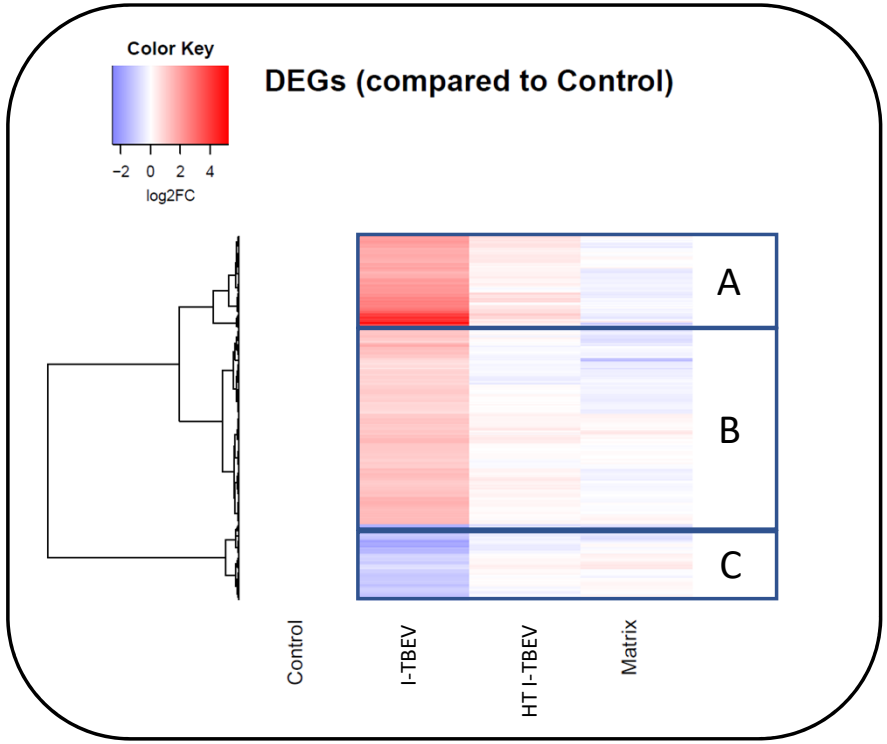
RNAseq identifies antiviral defense and IFN signaling as I-TBEV-induced pathways

3 clusters:

A. Genes **strongly upregulated** in I-TBEV-stimulated cells
[**ISG56, MxA**, Viperin, MDA5, CCL8, **CXCL10**] -> **antiviral defense, IFN signalling**

B. Genes **upregulated** in I-TBEV-stimulated cells
[selected chemokines/cytokines] -> **immunity**

C. Genes **downregulated** in I-TBEV-stimulated cells
[ribosomal proteins; lipid metabolism; ICAM-1, DC-SIGN, MHC-II, CD14]

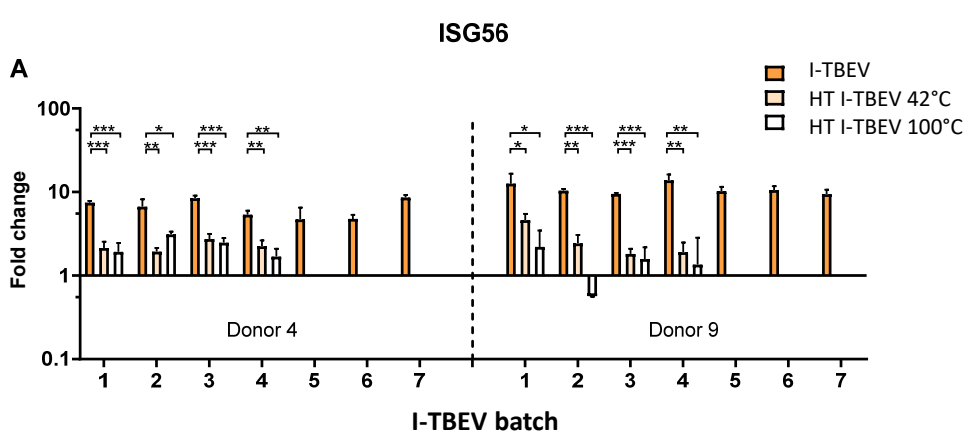


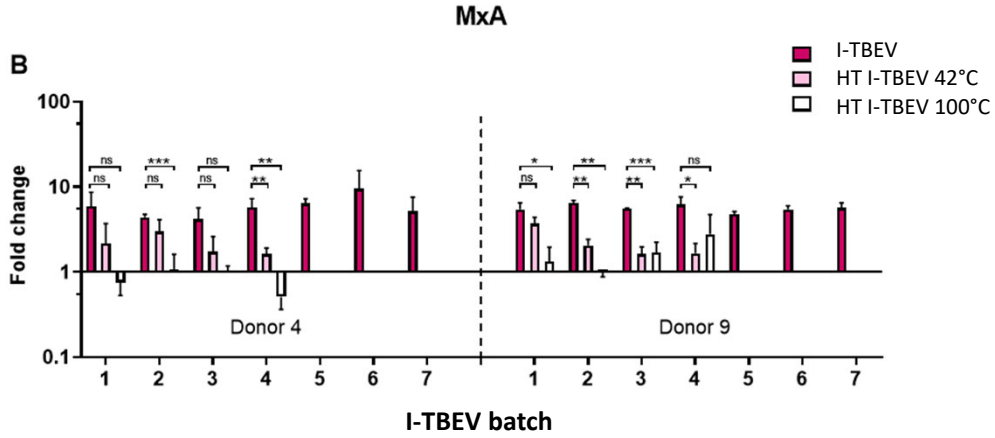
university of groningen

Signorazzi, Pennings, Etna, Noyam Coccia & Huckriede Vaccines 2021

umcg

PBMCs respond to different batches of I-TBEV in a consistent way



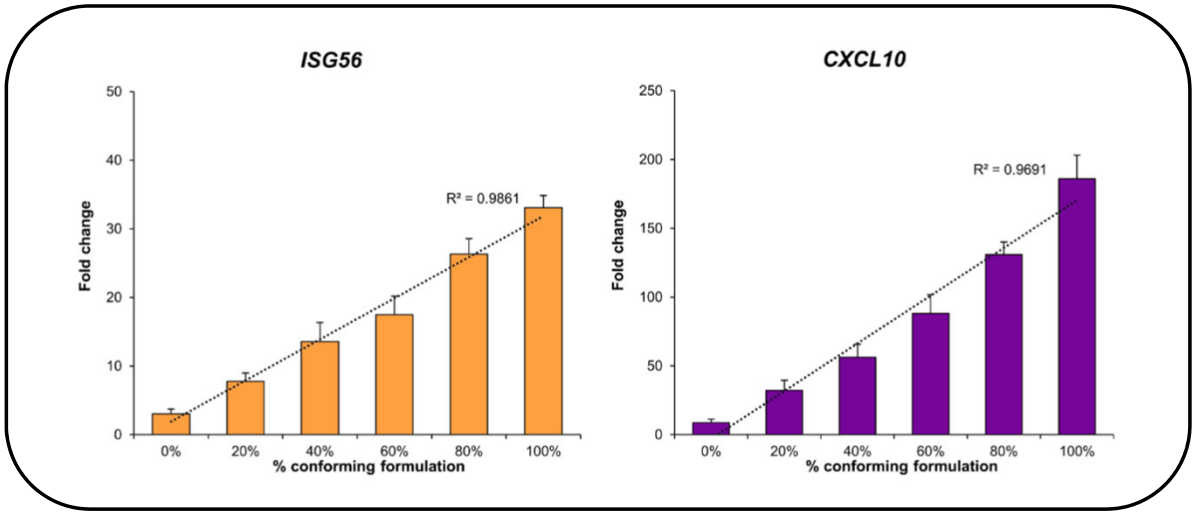


university of groningen

Signorazzi, Etna, Coccia & Huckriede ALTEX 2021

umcg

PBMCs detect the amount of conforming I-TBEV in a batch with high resolution



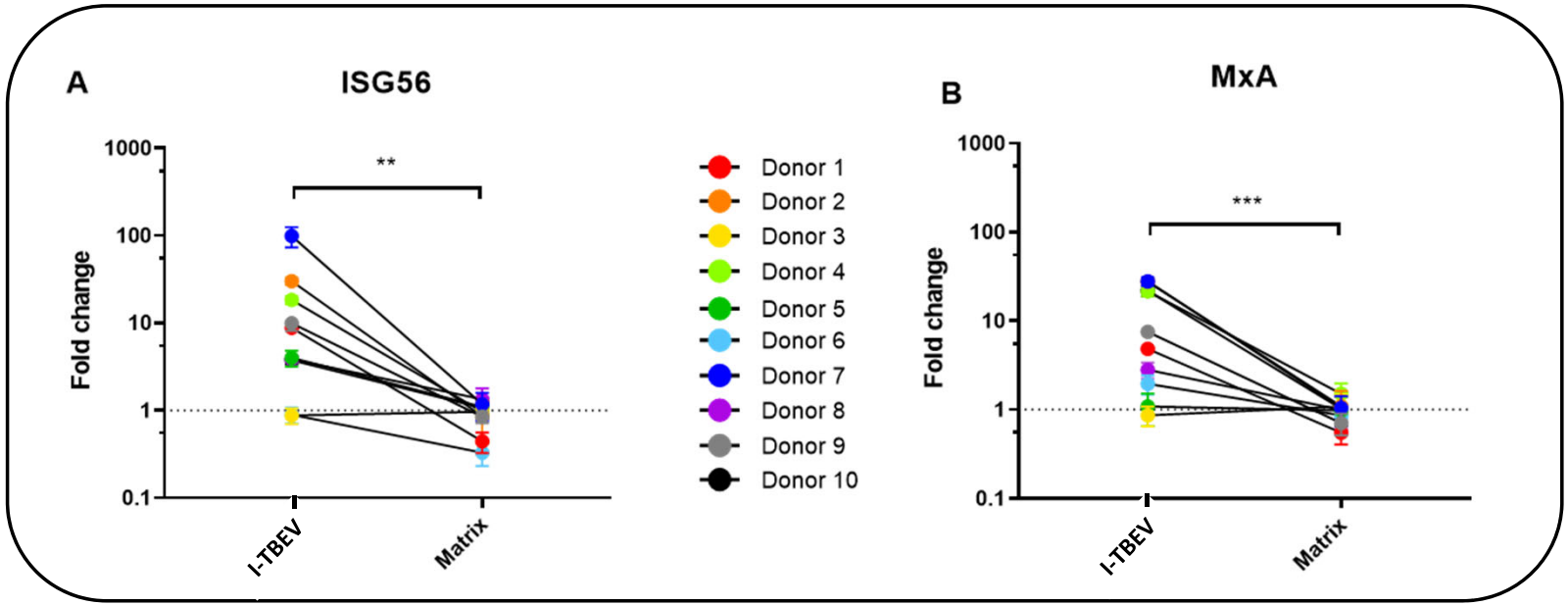
- Linear relationship between % of conforming product and response
- 20% difference results in statistically significant differences in response

university of groningen

Signorazzi, Etna, Coccia & Huckriede ALTEX 2021

umcg

PBMCs from different donors respond in a qualitatively similar manner (though quantitatively different)

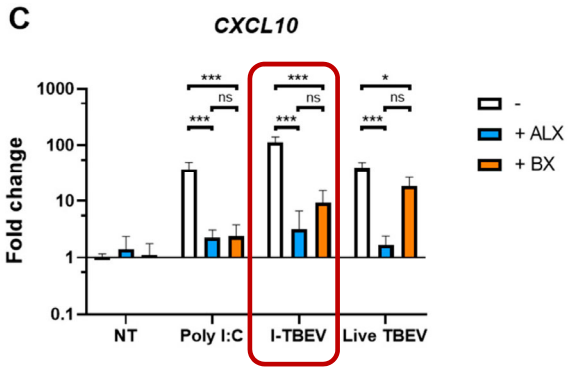


university of groningen

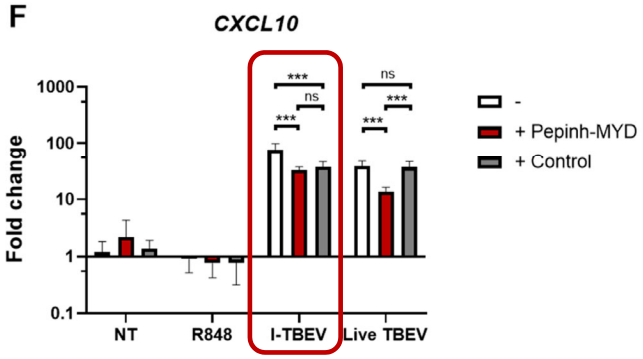
Signorazzi, Etna, Coccia & Huckriede ALTEX 2021

umcg

Activation of cryopreserved PBMCs relies on triggering of the RIG-I pathway



Inhibitors interfering with RIG-I pathway block I-TBEV induced responses



Inhibitors of TLR signaling have no specific effects

Signorazzi, Pennings, Etna, Noyam Coccia & Huckriede Vaccines 2021

Conclusions

- *In vitro* systems mimicking diverse aspects of the immune system are available
- Simple PBMC-based systems allow the study of vaccine-induced effects on DCs, conventional CD4 and CD8 T cells, and T follicular helper cells; B cells are currently under investigation
- DCs and T cells show distinct responses to different types of vaccines which correlate with responses *in vivo*
- PBMCs can detect differences in vaccine quality with high resolution and can be used in the context of batch quality control

ACKNOWLEDGEMENTS

- Aurora Signorazzi
- Gabriela Tapia Calle
- Shuran Gong
- Jacqueline de Vries-Idema
- Anke Huckriede

University Medical Center Groningen
Groningen, The Netherlands

- Eliana M. Coccia
- Marilena P. Etna

Istituto Superiore de Sanitá, Rome, Italy

- Jeroen Pennings

Nat. Inst. Public Health and the Environment
Bilthoven, The Netherlands

vac2vac

Innovative Medicines Initiative

university of groningen