National Nanotechnology Initiative

Swiss Federal Institute for Aquatic Science and Technology

International Network for Researching, Advancing, and Assessing Materials for Environmental Sustainability

2024 EU-U.S. NanoEHS Communities of Research (CORs) Workshop

October 16, Dubendorf (Zurich), Switzerland

Scaling up in complex systems (for NM safety testing):
Challenges when scaling up from *in vivo* to *in vitro*. Translating dosing regimen and other strains

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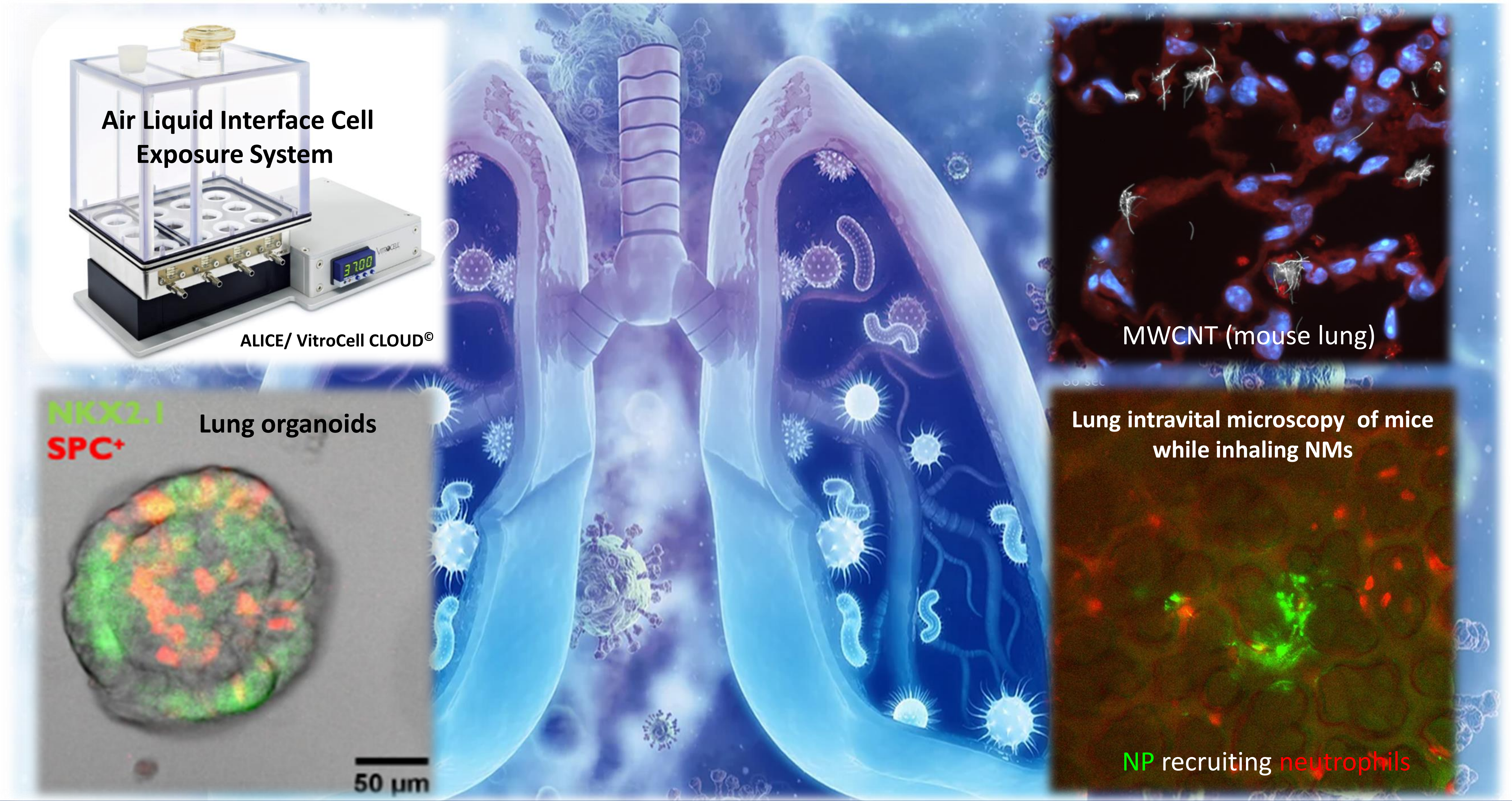
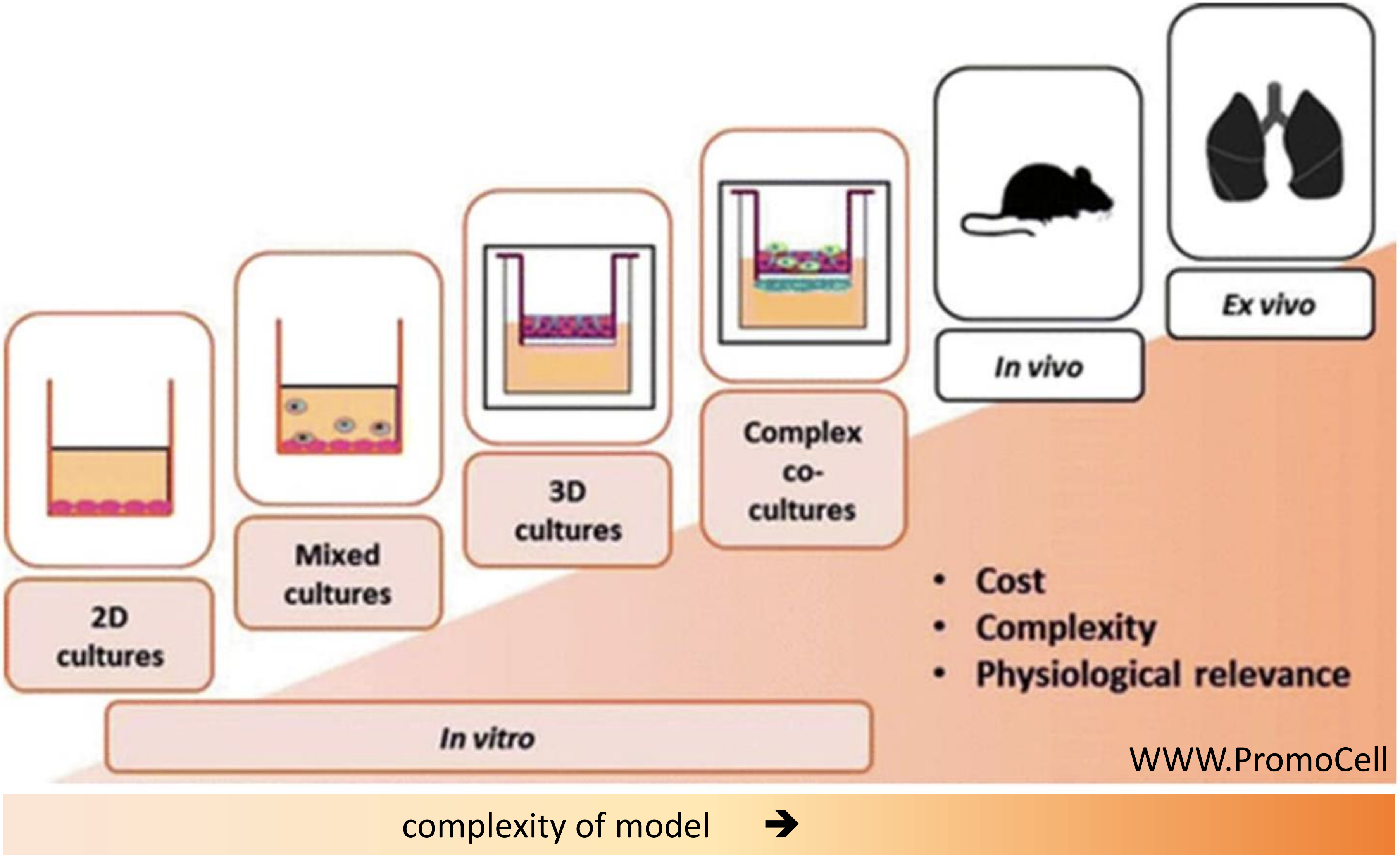
DZL Deutsches Zentrum für Lungenforschung



Tobias Stöger - Group Leader: Dynamics of Pulmonary Inflammation - Institute of Lung Health and Immunity (LHI)

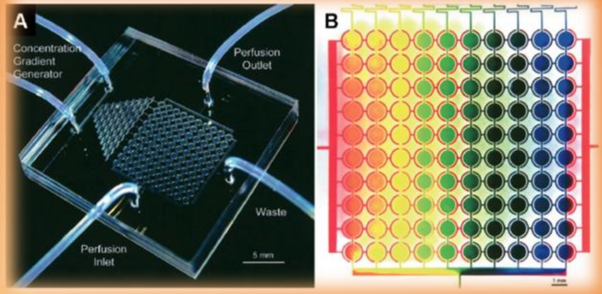
Scaling Up … for HTS / safety testing



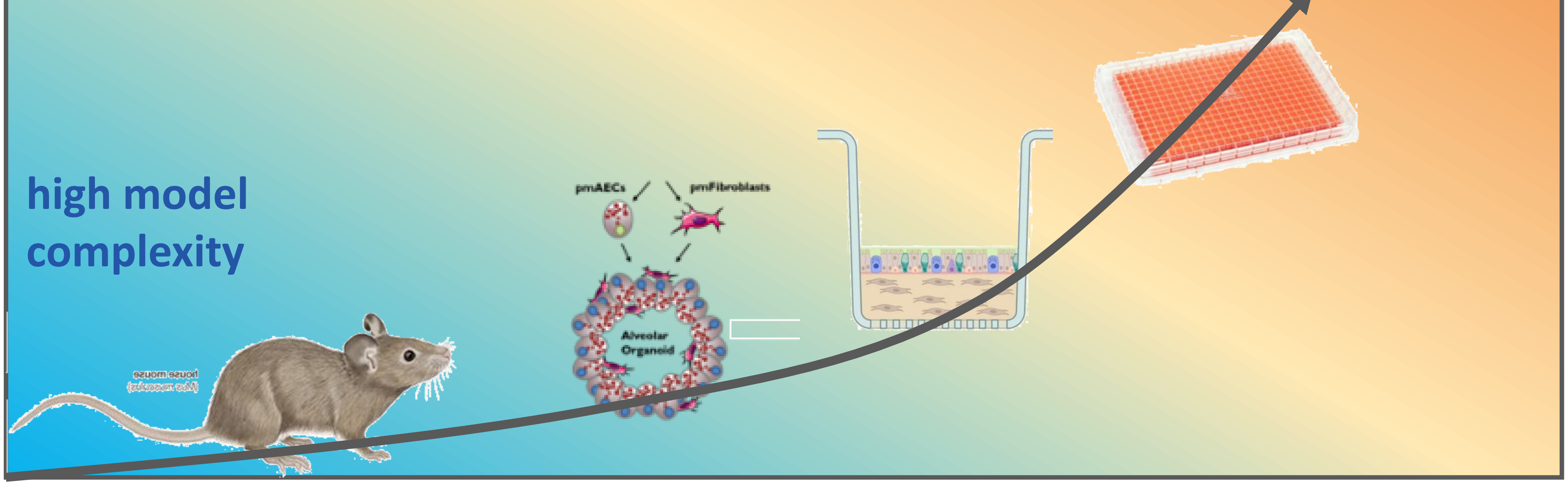
Scaling Up … for HTS testing / safety testing

Scaling up for High Throughput Screening requires detailed knowledge of the model:
- narrow window of investigation
- system-specific endpoints
- dynamic range of the dose response

high model specificity



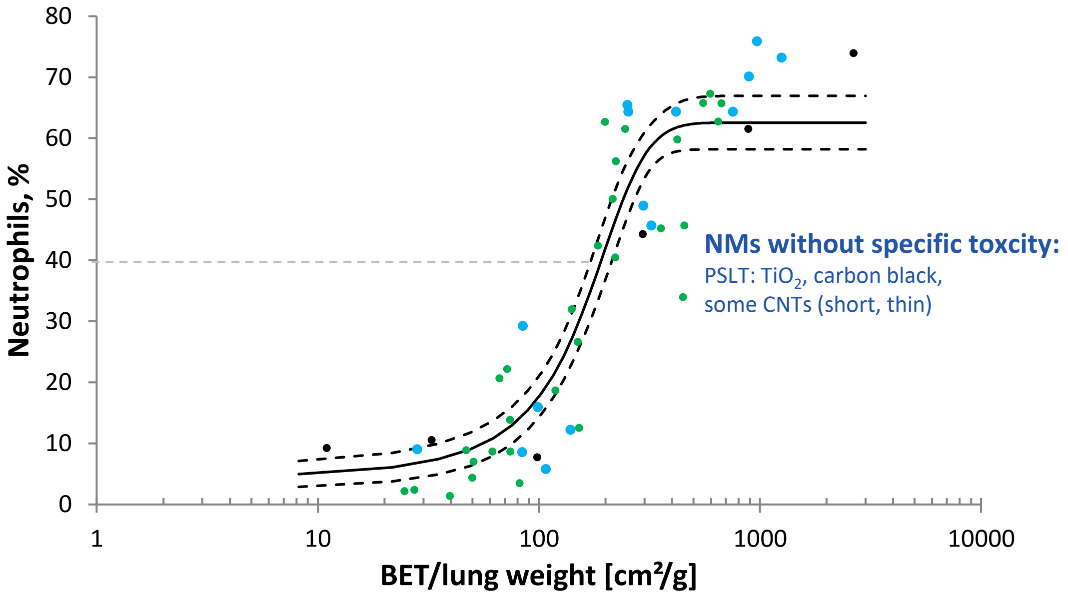
microfluidic cell culture array for high-throughput cell-based assays

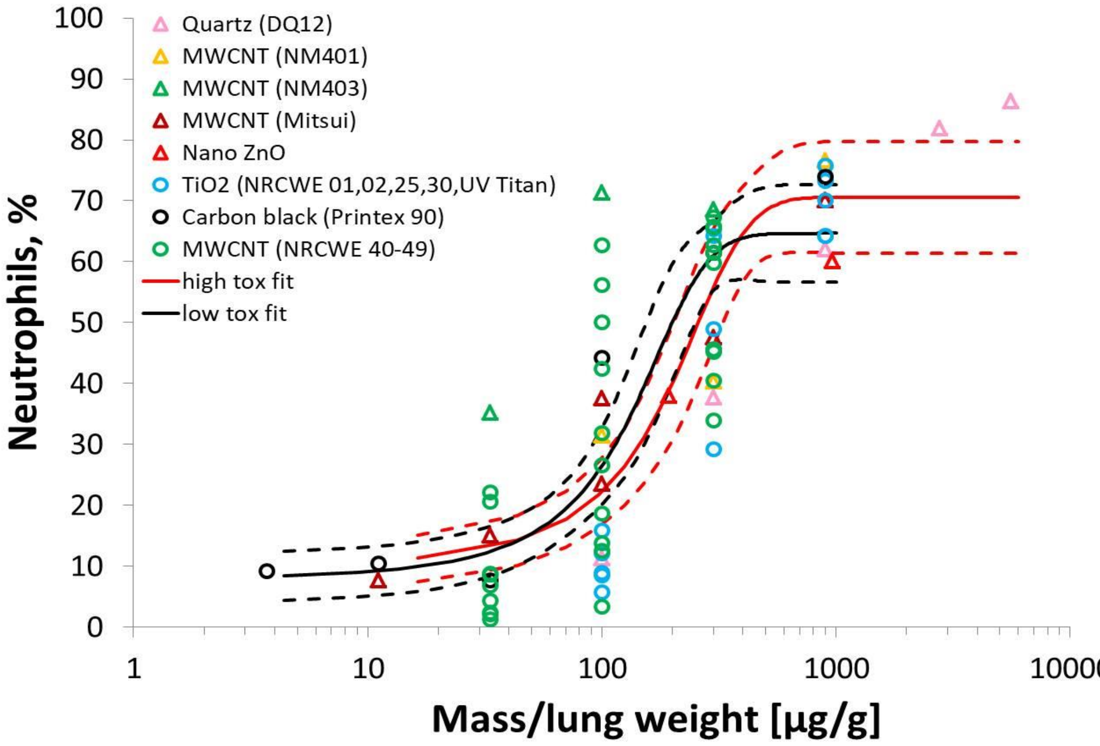


animal model | organoids | organotypic cultures | HTS-capable culture systems

Dose metric: **Particle surface area as driver of acute toxicity *(acute lung inflammation)* for PSLT particles**

Mouse data: Number of airspace neutrophils (broncho alveolar Lavage / BAL) 1d after instillation





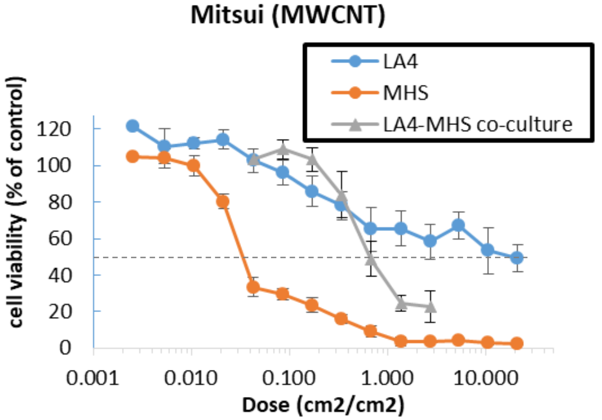
Danielsen, et al., Tox Appl Pharmacol, 386, 114830, 2020
Hadrup, et al., Vogel Nanotoxicology 13(9):1275-1292 2019

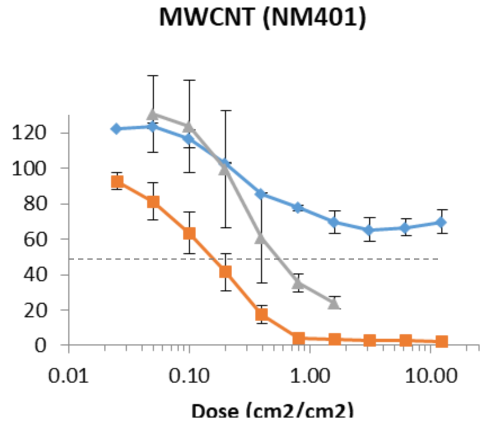
⇒ Surface area: allows for identification of Hazard Classes

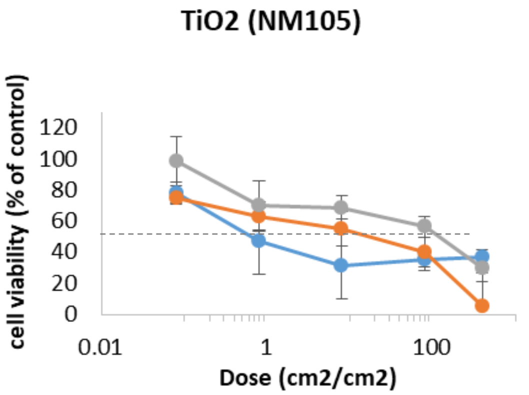
⇒ Hazard factors: 5 – 10-fold (relative to NMs without spec. toxicity / inflammation)

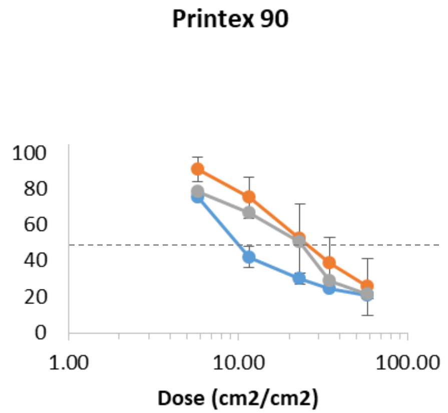
In Vitro toxicity ranking by deducing IC_{50} values

Submerged cell culture — LA-4: alveolar epithelial cells — MH-S: alveolar macrophages — LA-4 / M-HS cocultures

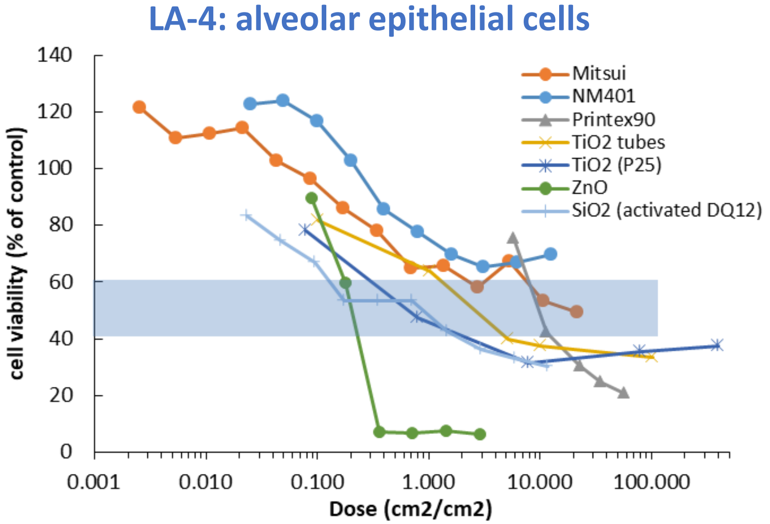




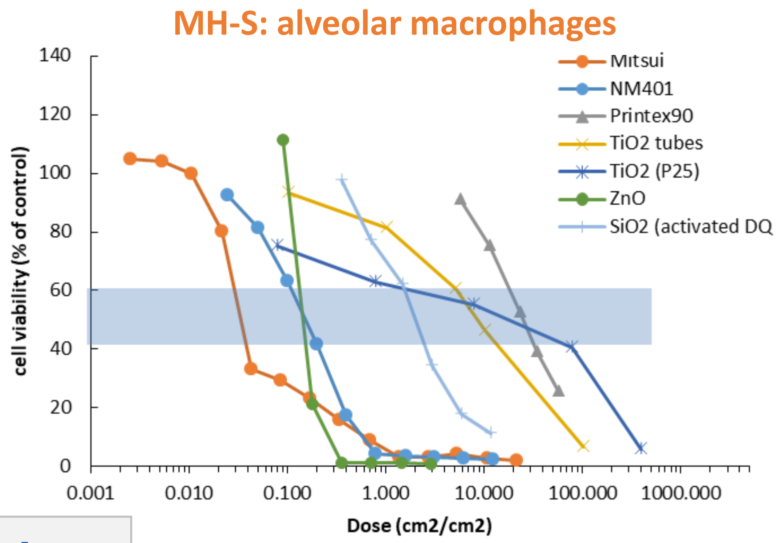




Dose corrected for deposited fraction



ZnO
DQ12
TiO_2 (P25)
TiO_2 (tubes)
MWCNT (Mits7)
CB (Ptx90)
NM401



MWCNT (Mits7)
NM401
ZnO
DQ12
TiO_2 (tubes)
CB (Ptx90)
TiO_2 (P25)

⇒ Cell type matters for high aspect ratio materials (HARN)

SmartNanoTox, https://cordis.europa.eu/project/id/686098/reporting

In vitro - In vivo comparison:

IC_{50} (cm^2/cm^2) for *in vitro* viability (WST1, 1d) and *in* vivo inflammation (neutro.; 1d +28)

		in vitro exposure			*in vivo* exposure		
Material	*In vitro* dep. fraction (murine, subm.)	Murine (submerged & ALI)			Mouse Instillation (applied dose)		Rat Inhalation (retained)
		LA-4 *(AT2)* subm.	MH-S *(Alv.M.)* subm.	LA4+ MHS (ALI)	1d	28d	28d
CNP (Ptx 90)	0.34	8.5	18	>20	0.07	1	1
MWCNT Mitsui-7	0.66	>10	0.03	0.30	0.005	0.05	0.02
MWCNT NM401	0.55	>10	0.15	0.12	0.006	0.01	0.02
ZnO (NM110)	0.60	0.2	0.3	0.3	0.008	---	---
Quarz (DQ12)	0.46	0.7	3	---	0.01	0.14	0.02

Inflammogenicity Classes
(4-fold hazard band)

Printex90: f_{haz} = 1

Low: f_{haz} = 1 - 4

Medium: f_{haz} = 4 - 16

High: f_{haz} = 16 - 64

Very high: f_{haz} = 64 - ….

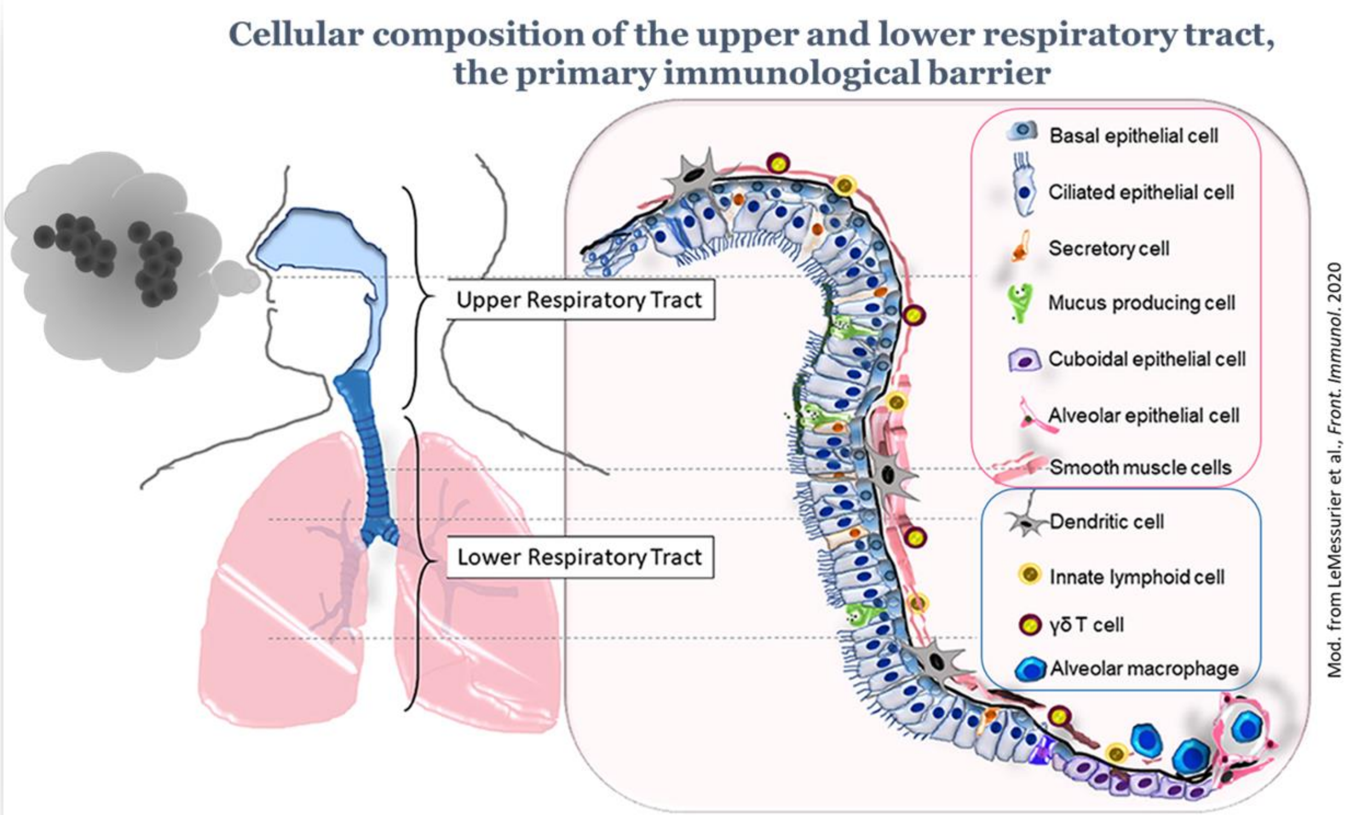
⇒ *in vitro cell viability is a reasonable predictor for in vivo toxicity particularly MH-S (phagocytes) for 28d (chronic toxicity)*

⇒ *in vitro cell viability (WST-1) requires higher dose than in vivo (BAL neutrophils): ~ 100-fold (1d), ~ 10-fold (28d)*

Which cells are exposed to inhaled particles?

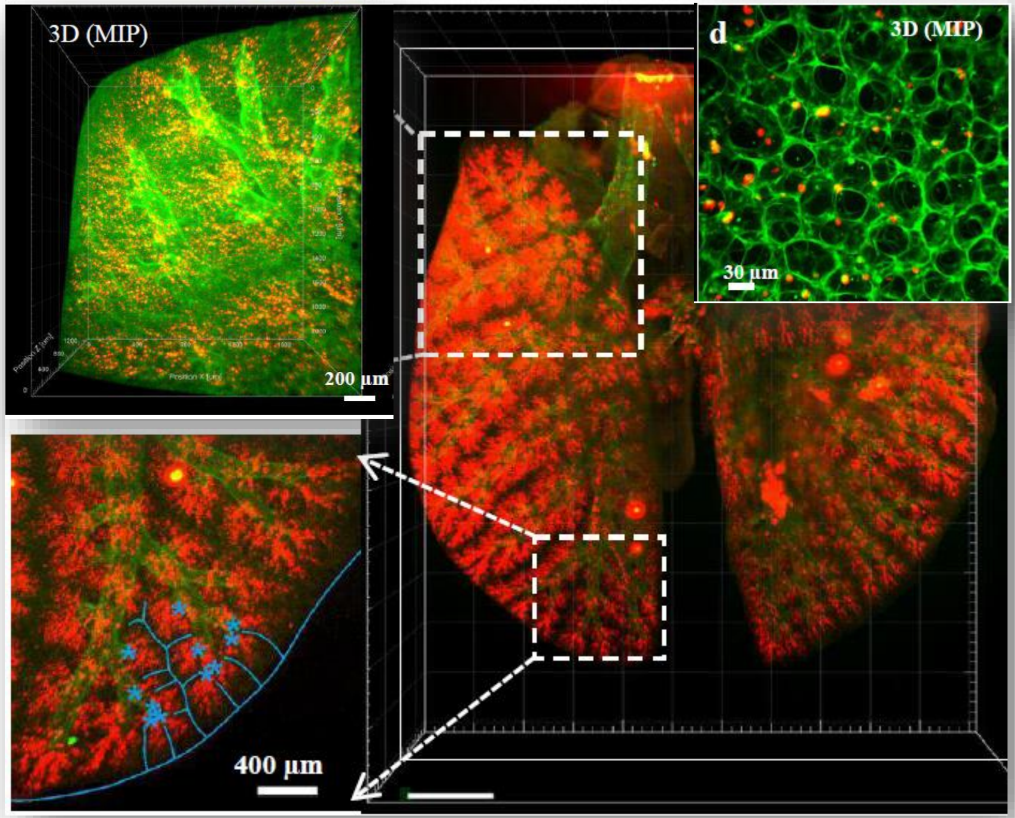
Particle deposition hotspots at the alveolar duct (proximal acinus)

Lin Yang
AG Schmid
@LHI



Mod. from LeMessurier et al., *Front. Immunol.* 2020

Deposition hot spots for inhaled particles



Lin et al., *ACS Nano*. 2019

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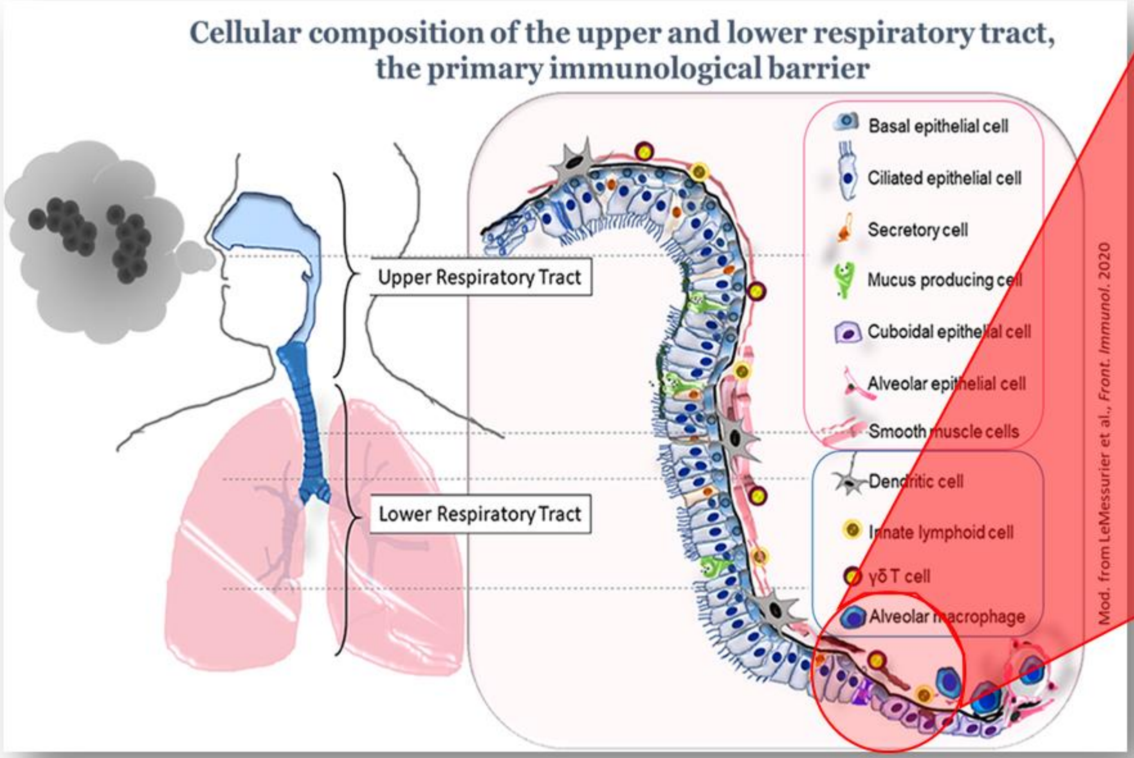
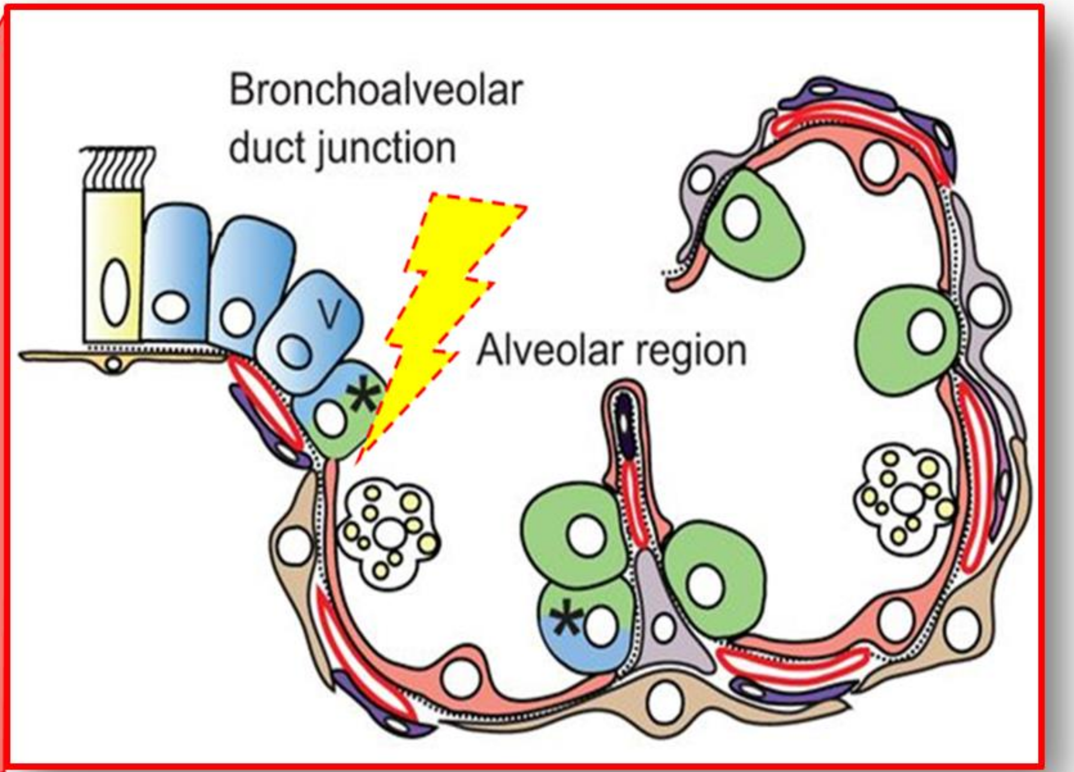
Deposition hot spots **for inhaled particles**





➔ 10-100 fold dose accumulation (ug/cm^2) at the alveolar duct

Which *lung cells* respond to nanoparticle inhalation and initiate or perpetuate lung inflammation?

human lung:

85 molecular cell types

33 'tissue' cells types:

15 epithelial cell types
airway, alveolar,…

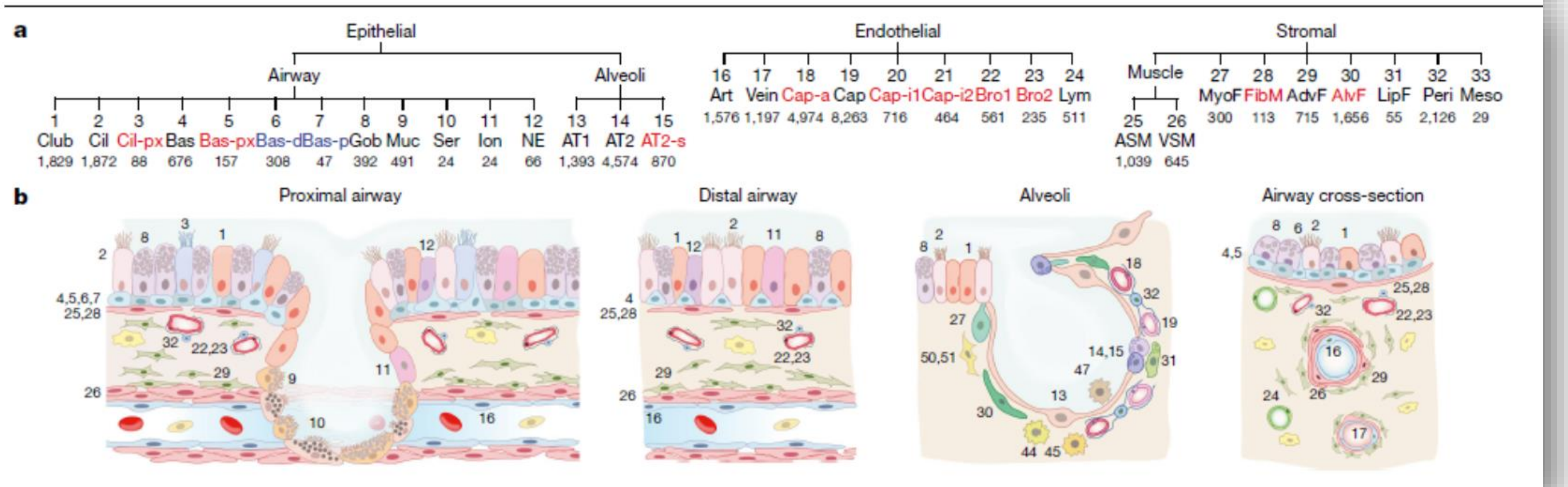
9 endothelial cell types
artery/vein, capillary, bronchiolar, lymphatic…

9 stromal cell types:
muscle, fibroblasts, mesothelial, …

Article

A molecular cell atlas of the human lung from single-cell RNA sequencing

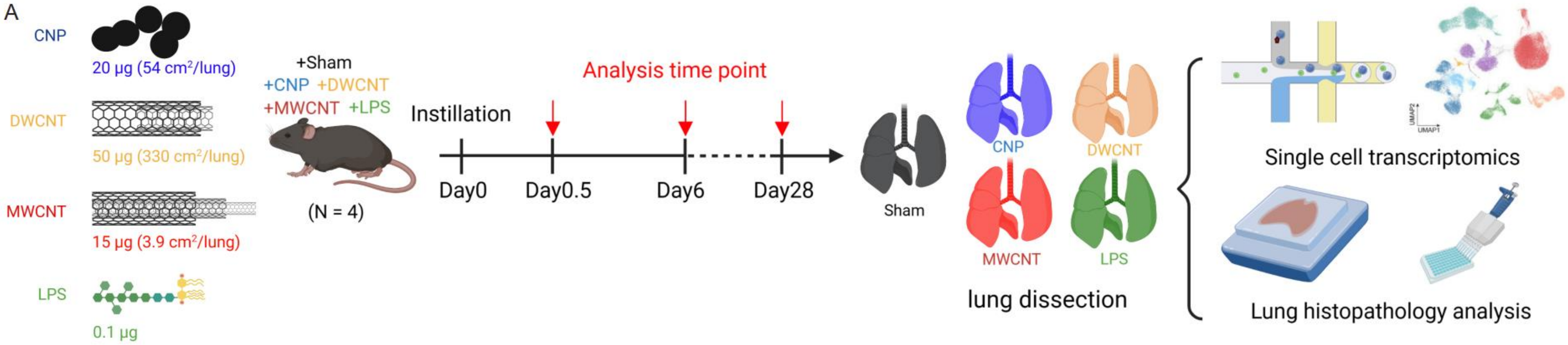
Travaglini et al., Nature, 2020



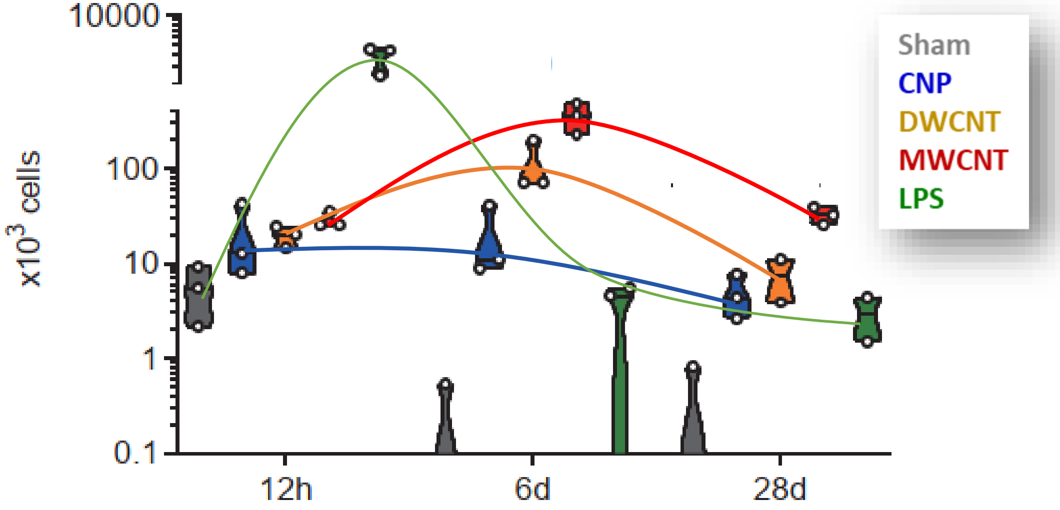
Single cell RNA sequencing of exposed mouse lungs to identify inflammation initiating key events (AOP)

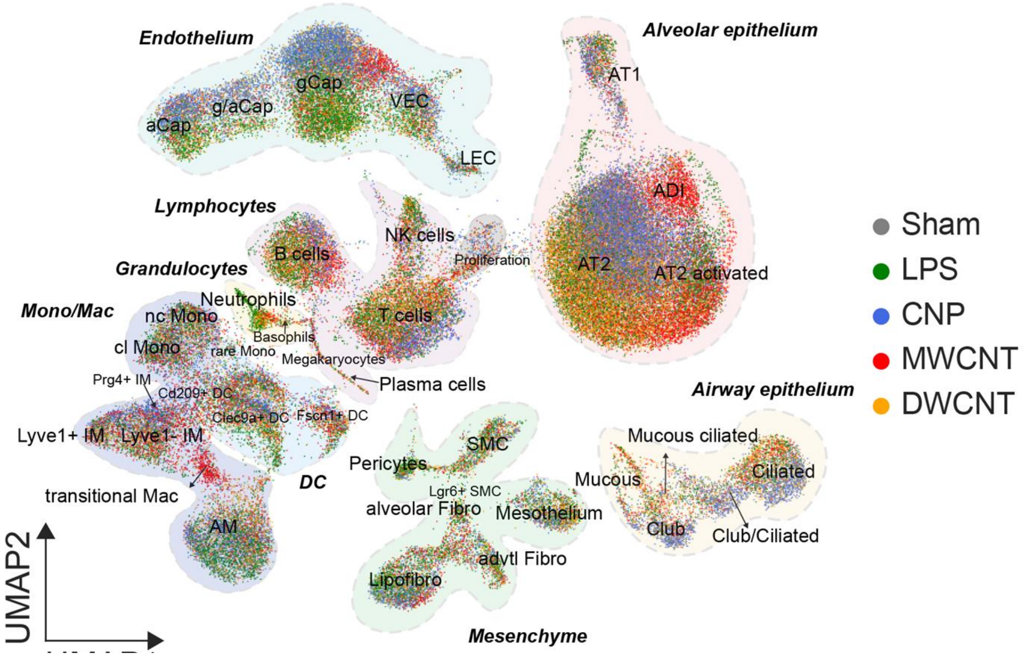
Herbert Schiller
Meshal Ansari
"THE scExperts"
@iLBD

Method



Inflammatory Response (airspace neutrophilia)





➔ **RNA sequencing of 100.000 cells from 64 mouse lungs identified 41 cell types/states**

Voss, Han, et al. in preparation & https://doi.org/10.1101/2024.02.10.579746

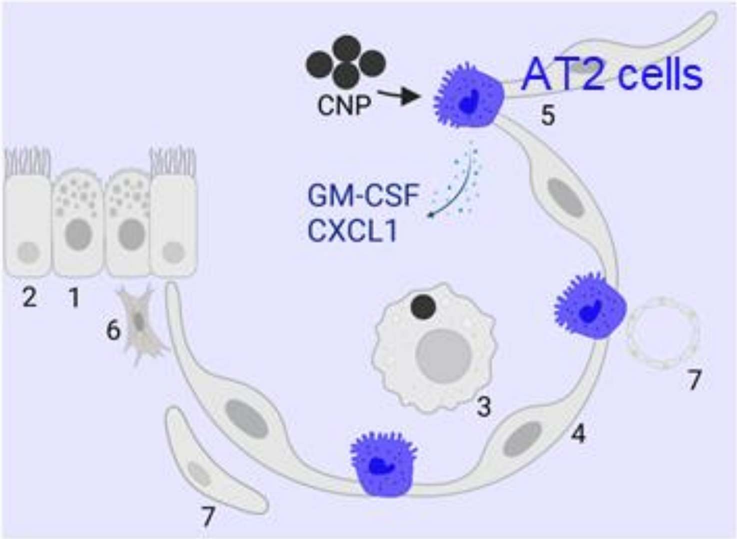
NM specific initiation of inflammation *(Mode of Action)*

… the cell type to be used for the *in vitro* testsystem matters!

Inflammation initiating / neutrophil cytokine releasing cell types revealed by scRNAseq and BAL cytokine profiling in mice

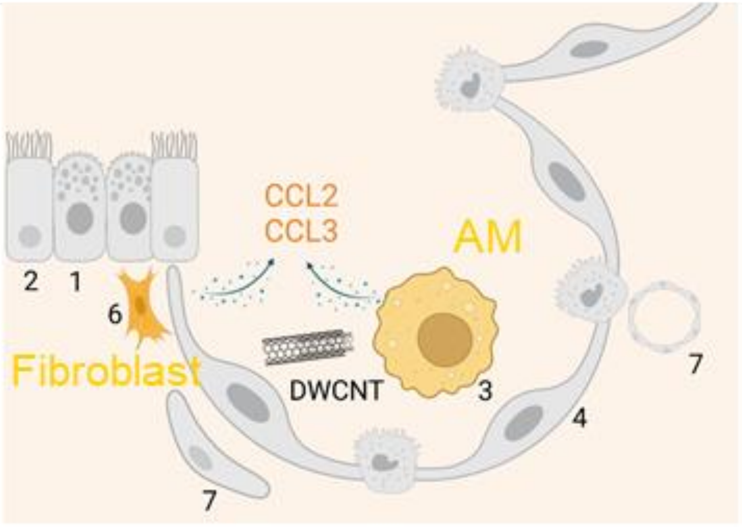
Lung cell types identified to release cytokines attracting inflammatory leukocytes:

Lung epithelial cells



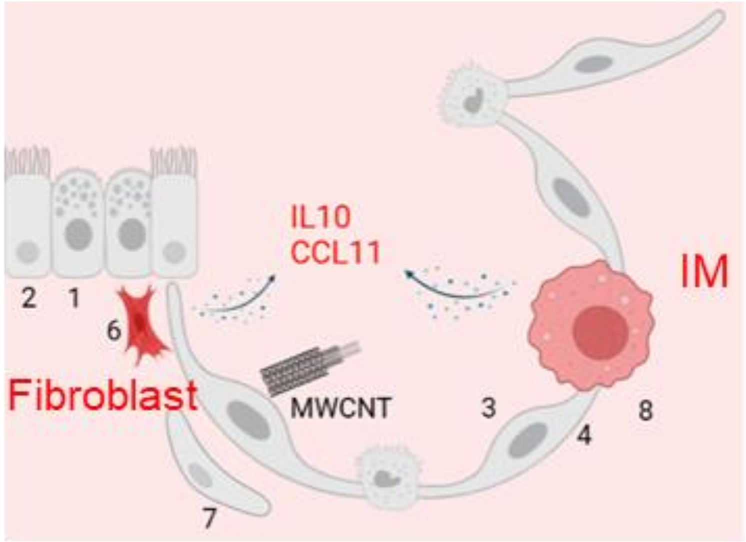
Neutrophil attraction

Alveolar macrophages & fibroblasts



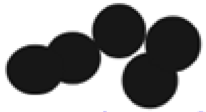
Neutrophil / monocyte attraction

Interstitial macrophages & fibroblasts



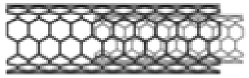
Th2 immunity /eosinophil trafficking

CNP



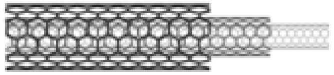
20 µg (54 cm²/lung)

DWCNT



50 µg (330 cm²/lung)

MWCNT



15 µg (3.9 cm²/lung)

Voss, Han, et al. in preparation & https://doi.org/10.1101/2024.02.10.579746

The HARMLESS Artificial Intelligence High-Throughput Screening Approach (AI-HTS) to Materials Safety Evaluation

Pekka Kohonen, Vesa Hongisto, Penny Nymark and **Roland Grafström**

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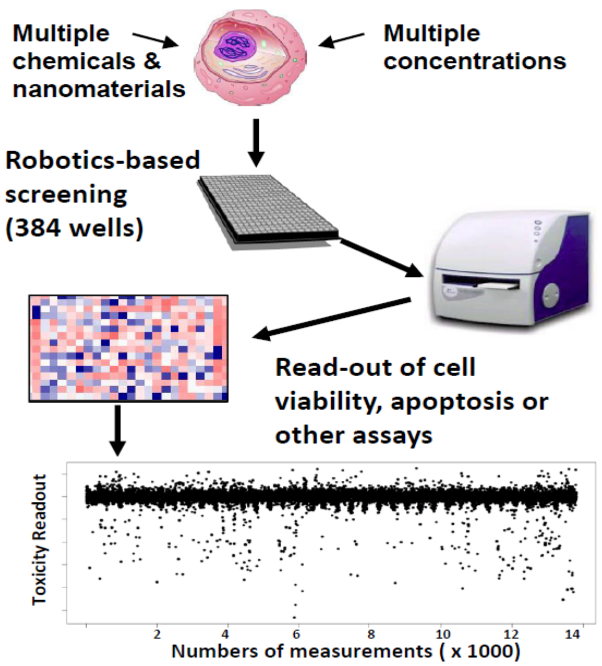
HARMLESS

Advanced High Aspect Ratio and Multicomponent materials:
towards comprehensive intelLigent tEsting and Safe by design Strategies

presented at Nanotox2024 (Venice)

HARMLESS

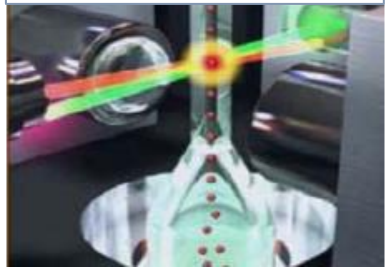
High-throughput systems toxicology for animal-free risk assessment



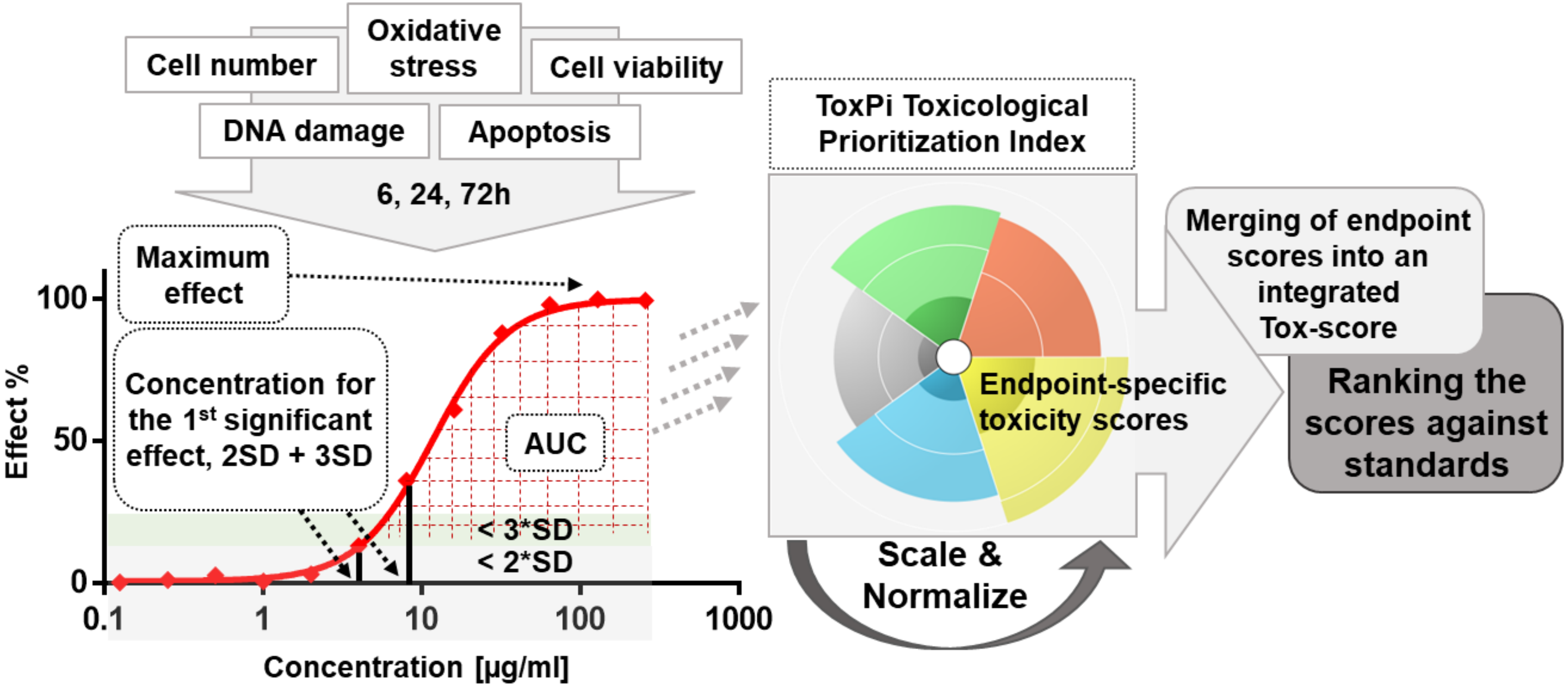
70+ materials based on “Tox5 scoring”/MoA analysis of dose response assessment

4 human cell line models:
BEAS-2B, A549, THP-I and HepG2

Predictive toxico-genomics space modeling:
- Differential expression, pathway analyses
- PTGS scoring/dose-response capture
- PTGS-based organ toxicity / cytotoxicity prediction using reference data, e.g., TG-GATEs, Connectivity map, and exposure e.g., therapeutic C_{max}
- PTGS component-based mode-of-action
- PTGS-MoA and AOPWiki annotations driven MIE/KE/AO and AOP analyses
- Integrative report, with AOP analysis, grouping and ranking based on potency and MoA effects of drugs/chemicals/materials

Traditional cell culture	High-throughput cell-based screening	RNA-sequencing	High throughput transcriptomics
			
Data points: 30-100	*~25000 /analysis*	*10-40 x10^6 /analysis*	*0.5-3 x10^6 /analysis*
coverage of one endpoint	**1-5 endpoints; *HT-ToxScore***	**genome-wide coverage**	**reduced feature set *(400-2500 genes)***

Misvik's "HT-Tox5 scoring" for MoA screening, hazard ranking and grouping of materials



"Tox5-score" is an *in vitro* toxicity scoring and ranking concept:

- Replaces one endpoint/timepoint concepts with a multi-time/endpoints score
- Covers growth, death, energy state, genotoxicity and reactive-oxygen species
- NM interference deducted (different assays/measurements, image gating, etc.)
- The metrics are combined/scaled/normalized using ToxPi software
- The output score is applied to potency ranking, hazard/severity estimates and grouping

Summary - scaling up for animal free testing strategies:

Material specific MoA require respective test systems

Approach 1. Complex cell based test systems to reproduce *in vivo* observed pattern

+ *in vivo* relevant by design
- low throughput

Approach 2. simple but HTS capable tests

+ high throughput
- data quantity needs to compensate for model quality

Respiratory toxicology: Consider unhomogenous NM distribution (deposition hotspots) when arguing for realistic dosing.

Thank you!

HELMHOLTZ RESEARCH FOR GRAND CHALLENGES

DZL Deutsches Zentrum für Lungenforschung

my and Markus group @LHI

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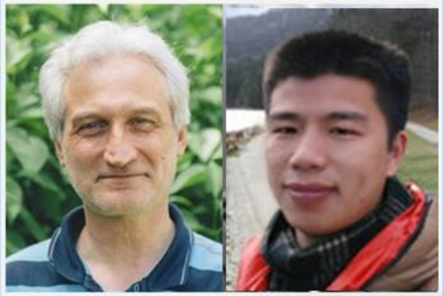

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CSC SCHOLARSHIP – A WAY TO YOUR DREAMS

BOW – Biogenic Organotropic Wetsuits

HARMLESS – Advanced High Aspect Ratio and Multicomponent materials: towards comprehensive intelLigent tEsting and Safe by design Strategies

SmartNanoTox – Smart Tools for Gauging Nano Hazards

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