SmartNanoTox
Smart Tools for Gauging Nano Hazards

SmartNanoTox International Online Conference

24 June 2020

Session 3 “In vivo - in vitro mapping”

HelmholtzZentrum münchen
German Research Center for Environmental Health

DZL
Deutsches Zentrum für Lungenforschung

HELMHOLTZ ASSOCIATION

SmartNanoTox

Smart Tools for Gauging Nano Hazards

Smart assays for AOP-based mechanism-aware toxicity assessment

Tobias Stoeger

SmartNanoTox International Online Conference - 24th June 2020

Session 3 "In vivo - in vitro mapping" 13.30 – 13.50

HelmholtzZentrum münchen
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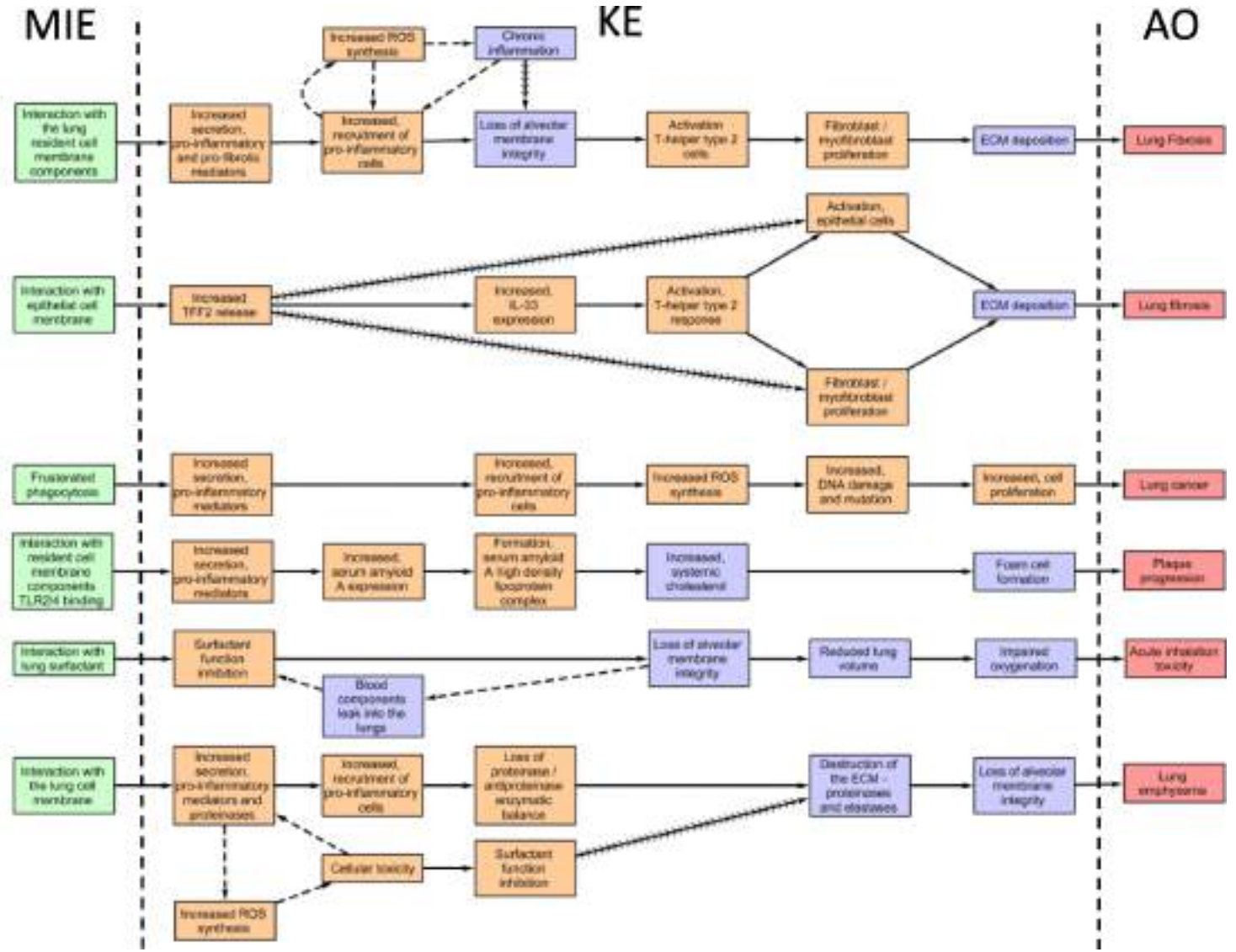
Smart assays for AOP-based mechanism-aware toxicity assessment



Halappanavar et al., P&FT, 2020

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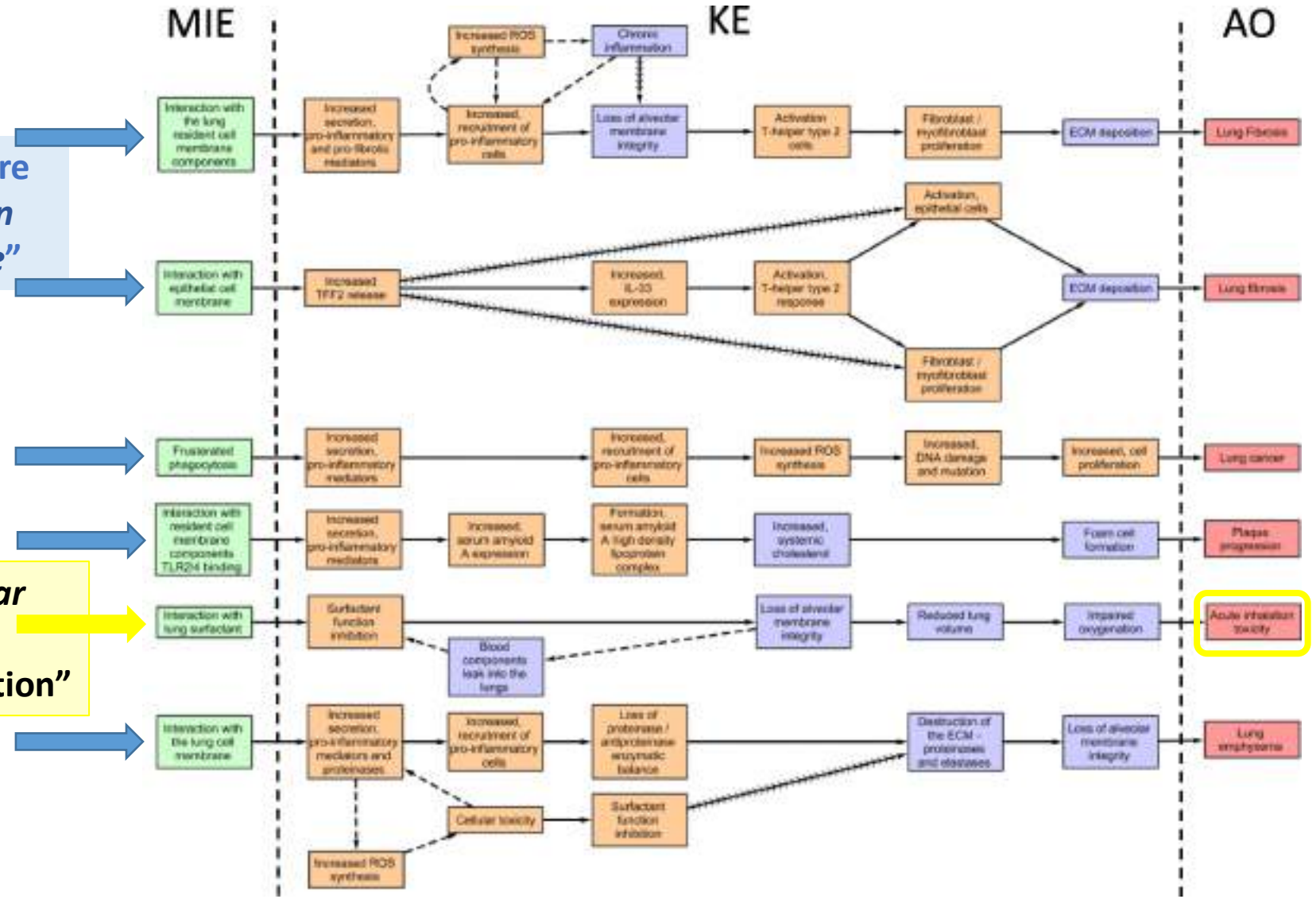
Smart assays for AOP-based mechanism-aware toxicity assessment

5 out of 6 AOPs share the MIE "*Interaction with cell membrane*"

Upstream of '*cellular interaction*': "Surfactant interaction"



Halappanavar et al., P&FT, 2020

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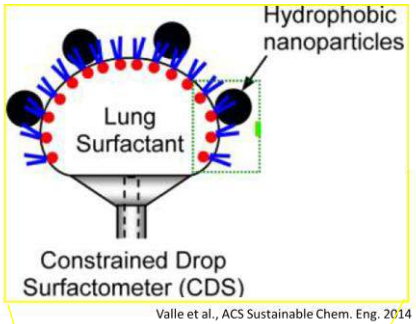
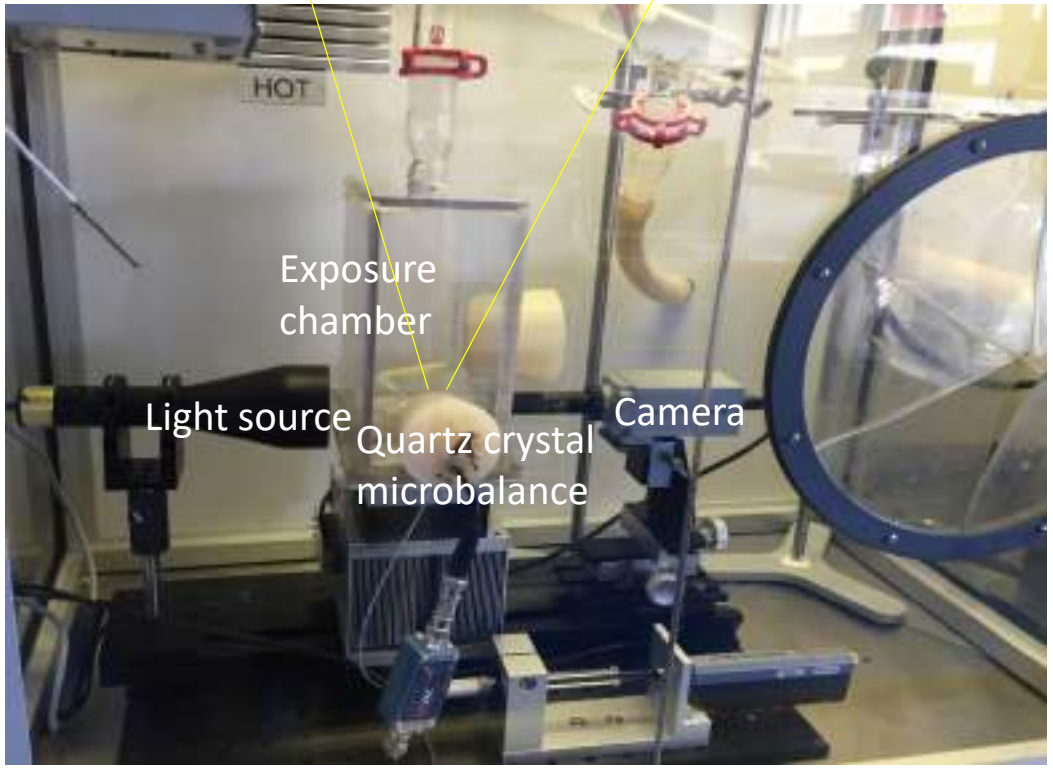
Lung surfactant inhibition, in vitro (Jorid B. Sørli, NRCWE)



Constrained Drop Surfactometer to test lung surfactant activity and inhibition under physiologically relevant conditions by in vitro simulation. Breathing is simulated by cycles of compressing and expanding the droplet using a motorized syringe.

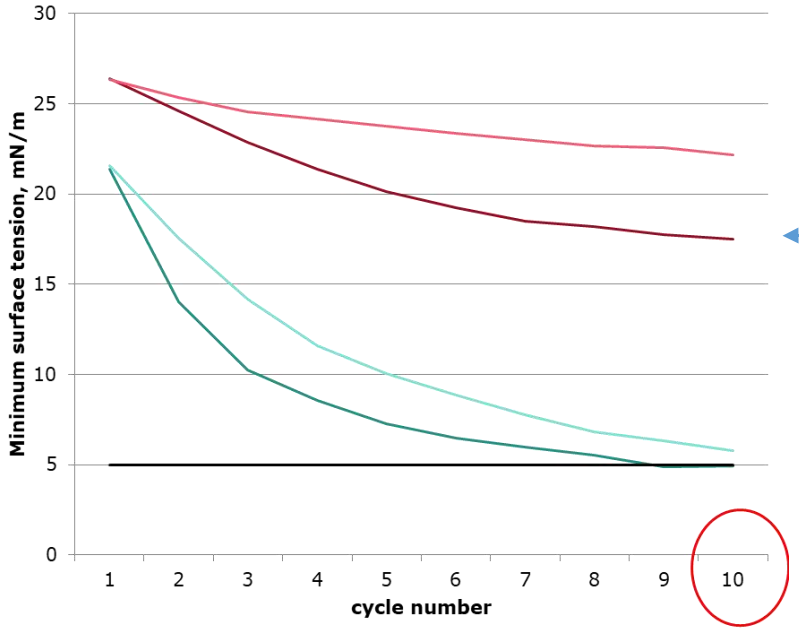
https://forsoegsdyrenes-vaern.dk/

Sørli et al., AJRCMB. 2016



Constrained Drop Surfactometer (BioSurface Instruments)

Test material and lung surfactant mixed, and function measured during first 10 cycles



Inhibitory concentration

Lung surfactant inhibition, in vitro (Jorid B. Sørli, NRCWE)

Material	Highlighted results
Quartz DQ12	
NM-200: Synthetic amorphous silicon dioxide	
NM-402: MWCNT	
NRCWE-051: SWCNT	
Printex90: Carbon black (14nm)	
Flammruss 101: Carbon black (95nm)	
NRCWE-058: graphene oxide	only carbon material affecting LS function
NRCWE-059: reduced graphene oxide	
NRCWE-001 , rutile TiO_2	
NRCWE-002, rutile TiO_2	
UV-Titan L181, rutile TiO_2	
NM-101, antase TiO_2	least inhibitory of TiO_2
NM-110, ZnO	most inhibitory of all tested materials
NM-111, ZnO coated triethoxycapryl silane	coating eradicate inhibitory effect of NM110
NRCWE-018, Fe_2O_3	

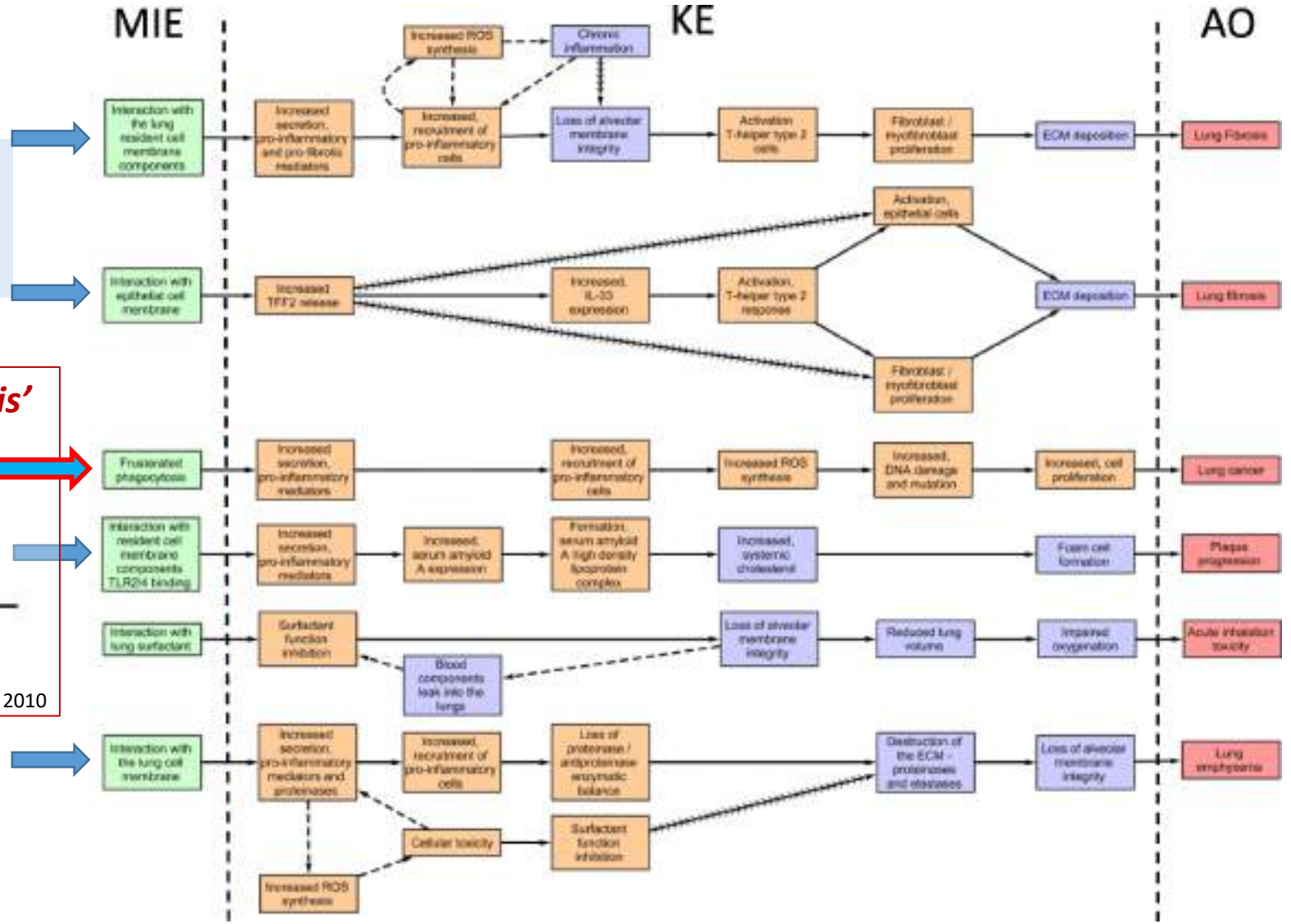
➔ **1st fully developed Smart Assay for 'Acute Inhalation Toxicity'**

Smart assays for AOP-based mechanism-aware toxicity assessment

5 out of 6 AOPs share the MIE "*Interaction with cell membrane*"

'Frustrated phagocytosis' **(material too long so that it cannot be fully internalized)**

Donaldson et al., Part Fibre Toxicol. 2010



Halappanavar et al., P&FT, 2020

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Macrophage fusion as surrogate for ‘Frustrated phagocytosis’

Olivier Joubert
Univ. Lorraine

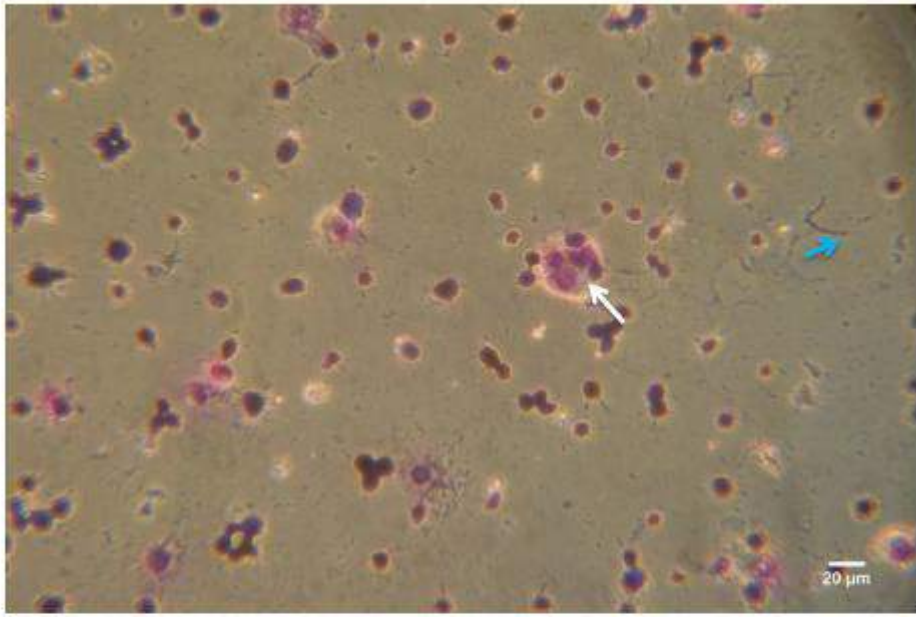
May–Grünwald–Giemsa stain (light microscopy x400)

Exposure of NR8383 rat alveolar macrophages to 12µg/mL of NRCWE 006 for 24h

Blue arrows: CNT

White arrows: macrophage *syncytia (macrophage fusion)*

(Comparison with long and thin CNT (NM 401) in progress)



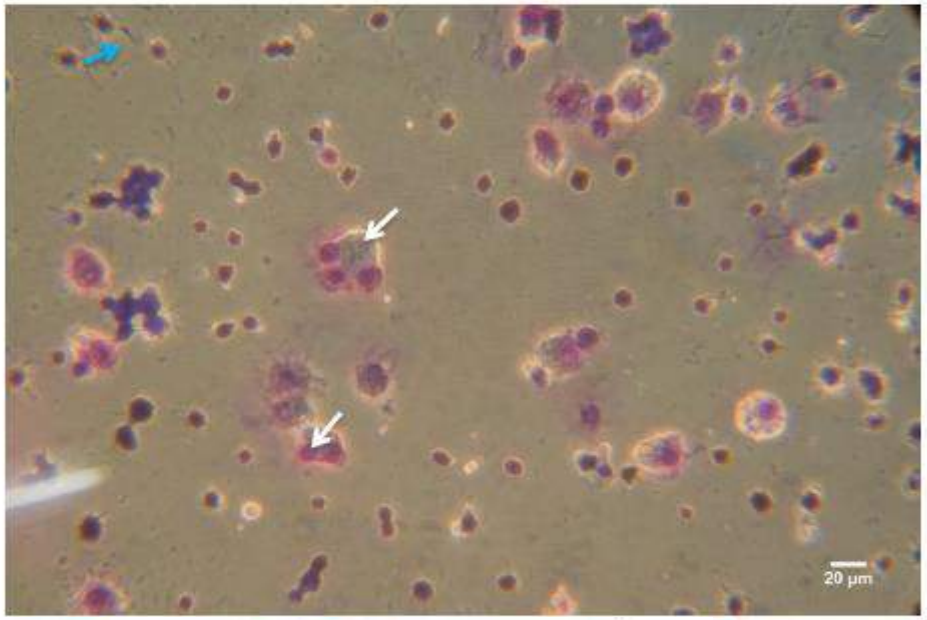


Figure 15 : Photographie de microscopie optique (X400) de macrophages NR8383 après traitement de 24h à NRCWE-006 à 12µg/ml. Les cellules déposées sur une lame de verre ont été fixées et colorées selon la technique de Giemsa. On peut observer les CNT (flèches bleues). Parmi les cellules vivantes on peut observer également la présence de syncytia (flèches blanches).

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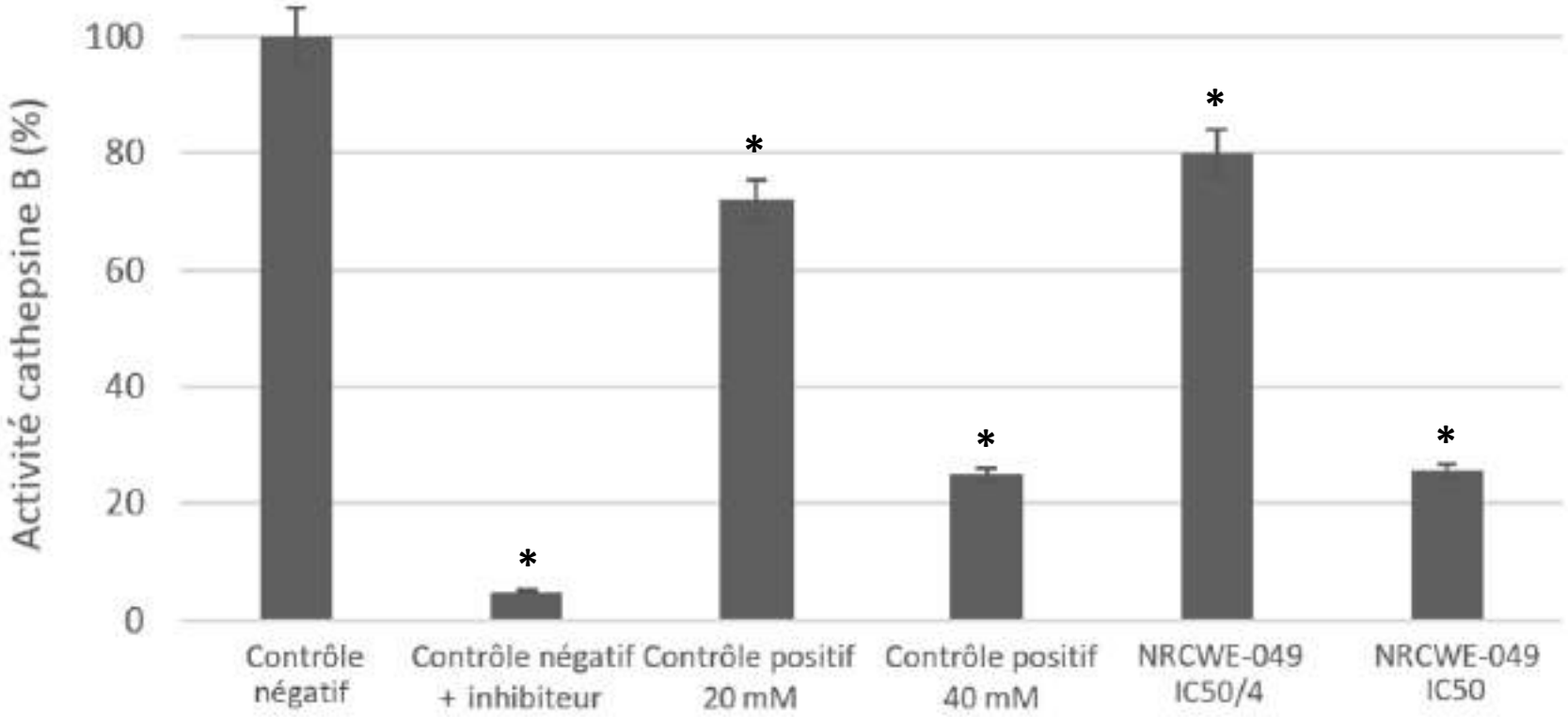
NM interaction with lysosome membranes - Cathepsin B assay

Olivier Joubert
Univ. Lorraine

Cytoplasmatic Cathepsin B activity as indictor for lysosomal membrane destabilization



→ CNT-NH2 seem able to permeabilize lysosomal membranes

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Cytokine profiling to detect '*secretion of inflammatory mediators*'

Olivier Joubert
Univ. Lorraine

Protein supernatant levels of NR8383 rat alveolar macrophages exposed to different CNTs at doses of ¼ IC50 *(metal oxides in progress)*

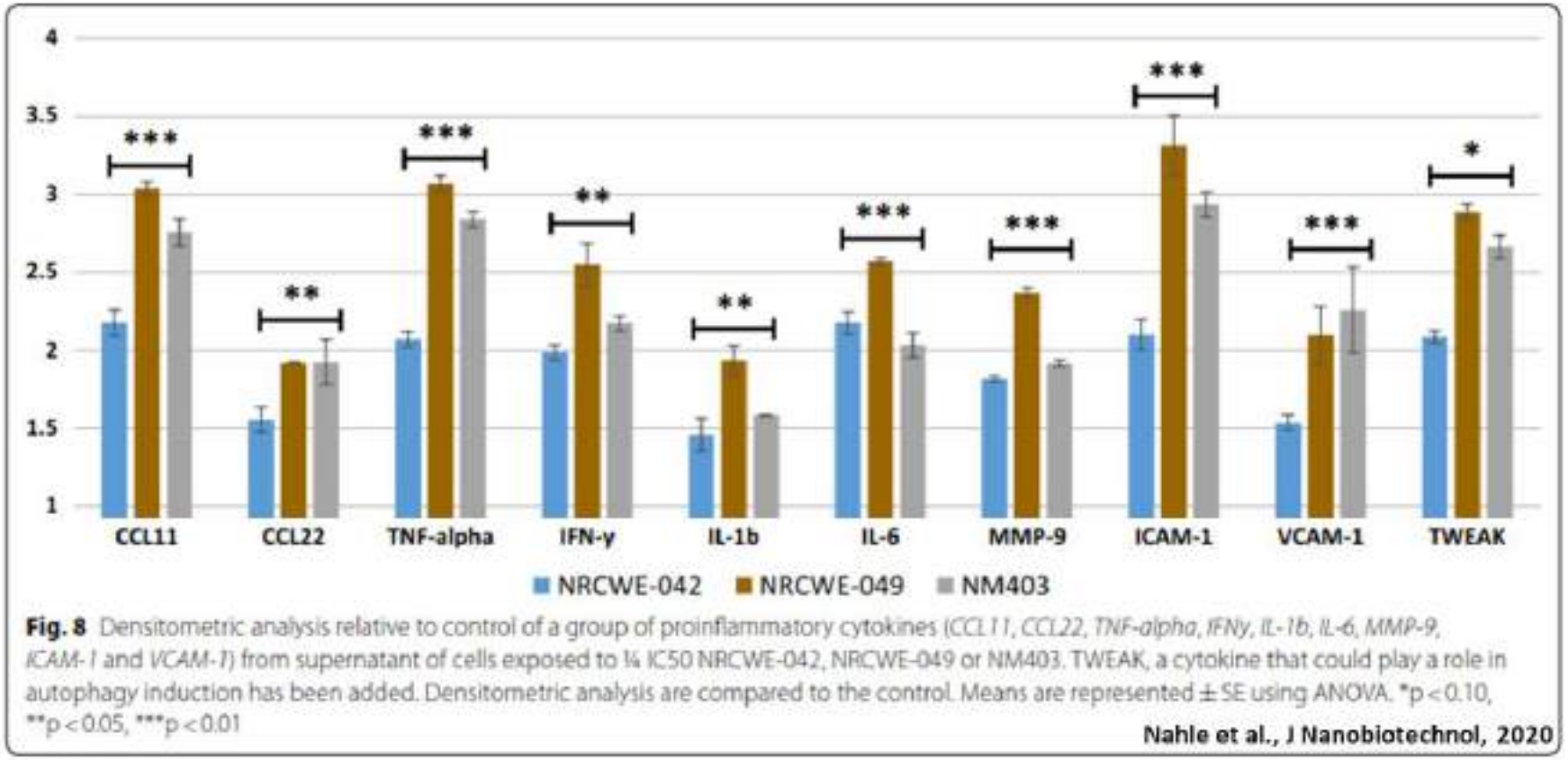

Fig. 8 Densitometric analysis relative to control of a group of proinflammatory cytokines (*CCL11, CCL22, TNF-alpha, IFNγ, IL-1b, IL-6, MMP-9, ICAM-1* and *VCAM-1*) from supernatant of cells exposed to ¼ IC50 NRCWE-042, NRCWE-049 or NM403. TWEAK, a cytokine that could play a role in autophagy induction has been added. Densitometric analysis are compared to the control. Means are represented ± SE using ANOVA. *$p < 0.10$, **$p < 0.05$, ***$p < 0.01$

Nahle et al., J Nanobiotechnol, 2020

Inflammatory potency CNT-COOH < CNT-X < CNT-NH_2

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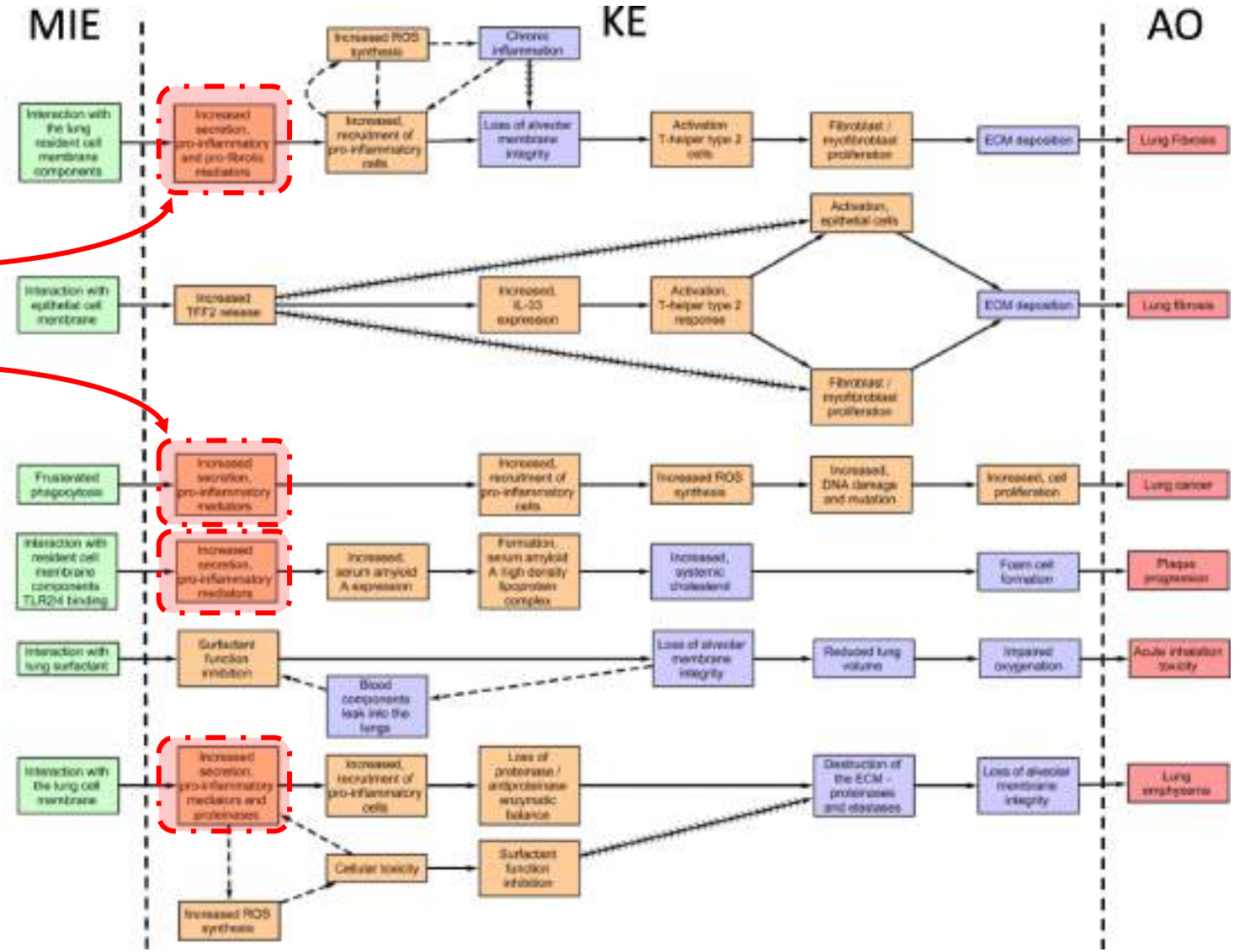
Smart assays for AOP-based mechanism-aware toxicity assessment

4 out of 6 AOPs share the KE:

Increased secretion of pro-inflammatory mediators



Halappanavar et al., P&FT, 2020

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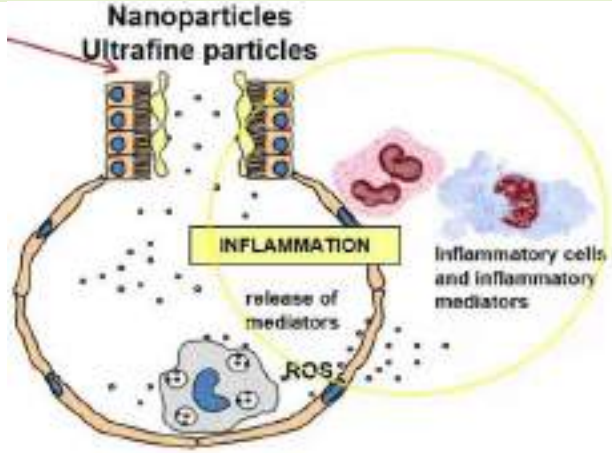
Secretion of pro-inflammatory mediators from lung cells

? Question : **Which cell type is initiating the *Molecular Initiating Event* (MIE) or Executing the *Key Event* (KE) to start the inflammatory response? Which cell type has to be used for the *Smart Assay*?**



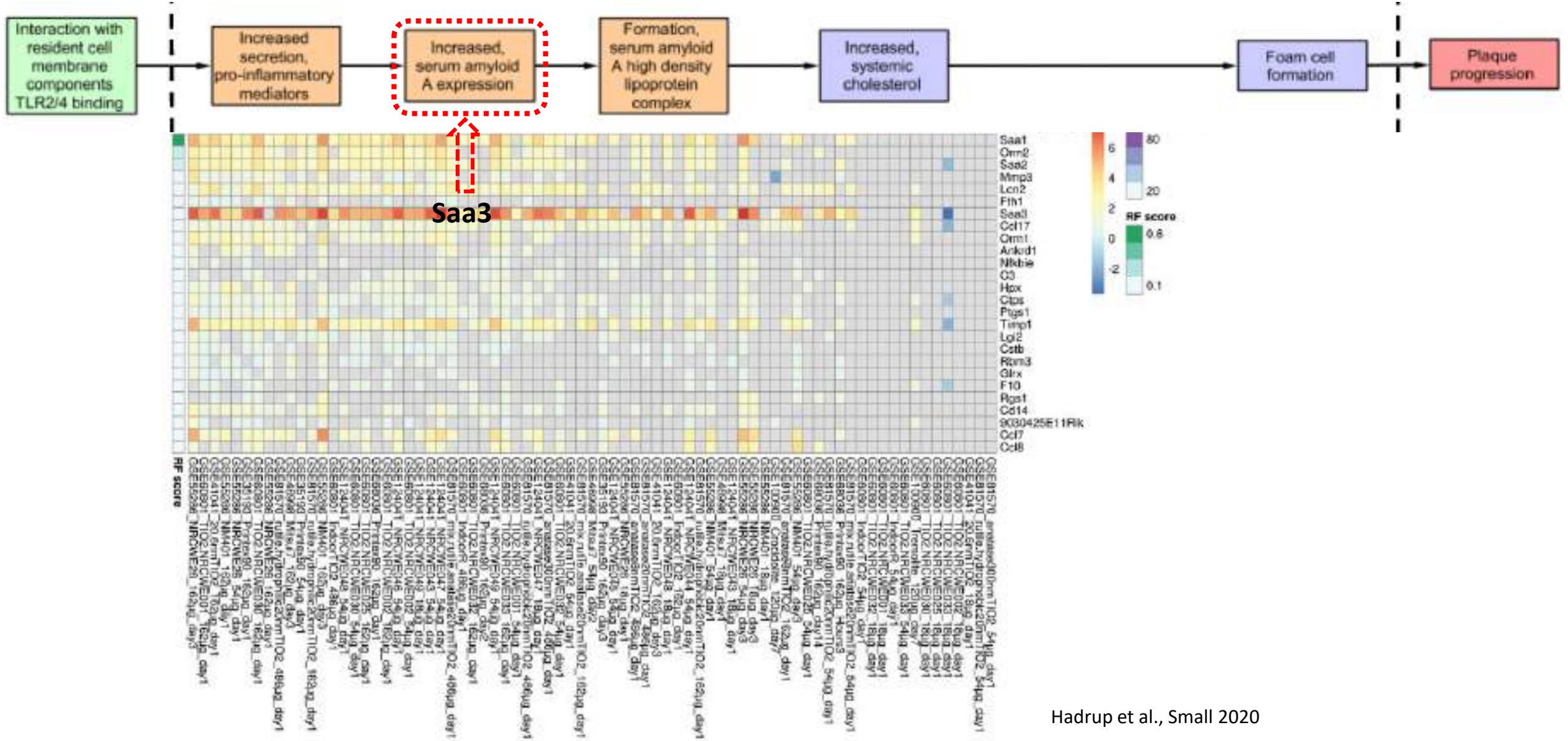
Mouse *in vitro* results:

In murine alveolar epithelial cells (LA4) and alveolar macrophages (MHS) and at doses up to IC50 the NMs: **DQ12, ZnO/NM110, MWCNT/NM401, MWCNT/Mitsui7, DWCNTs** and **CB/Printex90** failed to cause a significant release for any of the cytokines: **CXCL1, -2, -5, TNFα, IL-1β and IL-33**

In murine bone marrow derived macrophages (Ana1) doses of 60% viability **NO inflammatory signature** (Affymetrix, >2fold) is detected 3h, 9h and 24h after exposure to **CB/Printex90** and only a very moderate one for **DWCNTs** (Mmp12: x7, Il1rn: x4, Rgs1 x3). However after 30min treatment, ROS (cellular DCFH) and MAPK-signaling (p38, ERK1/2) are significantly induced.

In search of the pro-inflammatory mediator releasing lung cell type

scRNA sequencing of mouse lungs exposed to LPS, CB/Ptx90, MWCNT/Mitsui7 and DWCNT, 12h and 6 and 28days after exposure



Hadrup et al., Small 2020

In search of the pro-inflammatory mediator releasing cell type

Carola Voss Herbert Schiller

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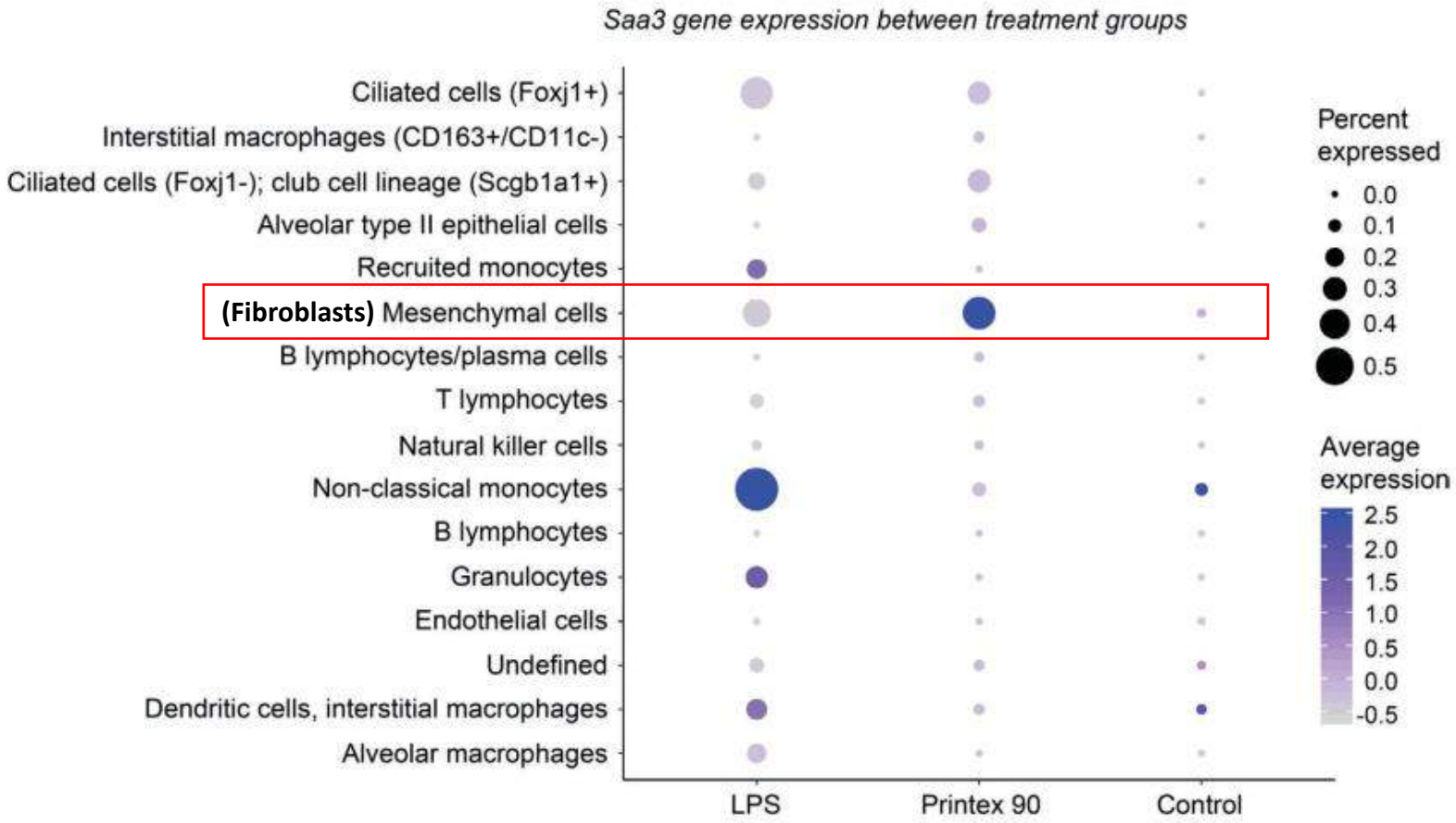
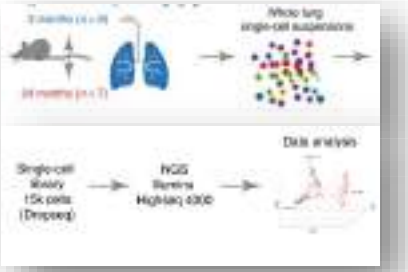
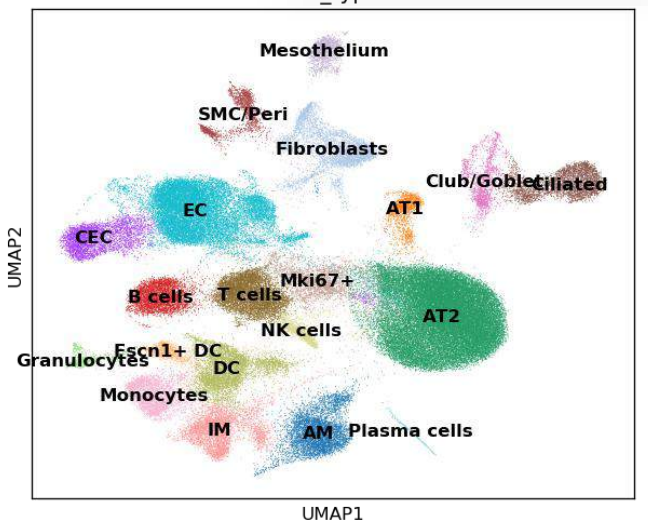






➔ **Unexpected role for lung fibroblasts in the acute phase response to NMs**

Hadrup et al., Small 2020

Secretion of pro-inflammatory mediators from epithelial lung cells

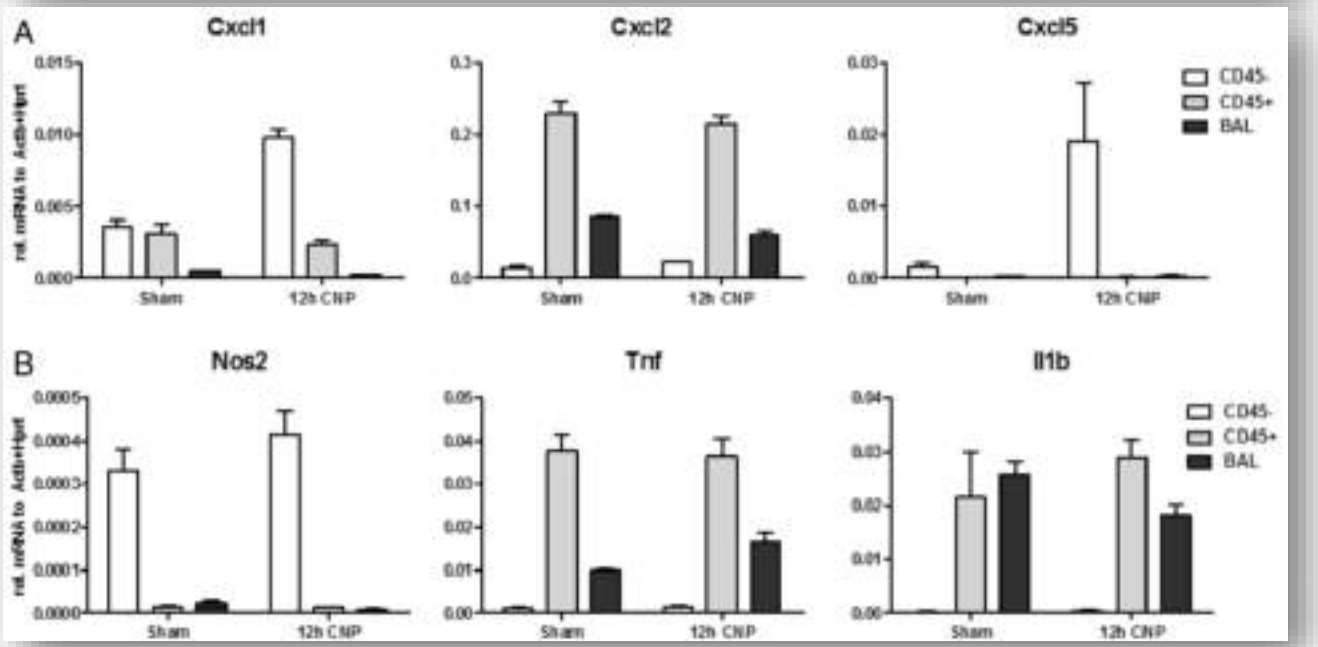
Chen et al. Particle and Fibre Toxicology (2016) 13:33
DOI 10.1186/s12989-016-0144-6

Particle and Fibre Toxicology

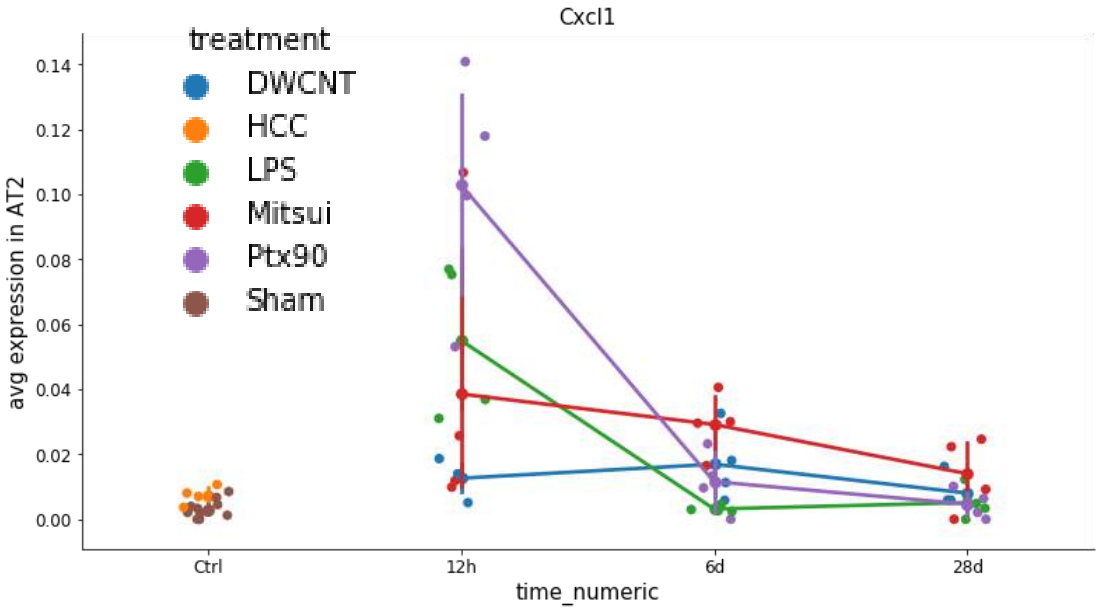
RESEARCH Open Access

No involvement of alveolar macrophages in the initiation of carbon nanoparticle induced acute lung inflammation in mice

Shanze Chen[1,2†], Renfu Yin[1,3†], Kathrin Mutze[1], Youjia Yu[1], Shinji Takenaka[1], Melanie Königshoff[1] and Tobias Stoeger[1*]



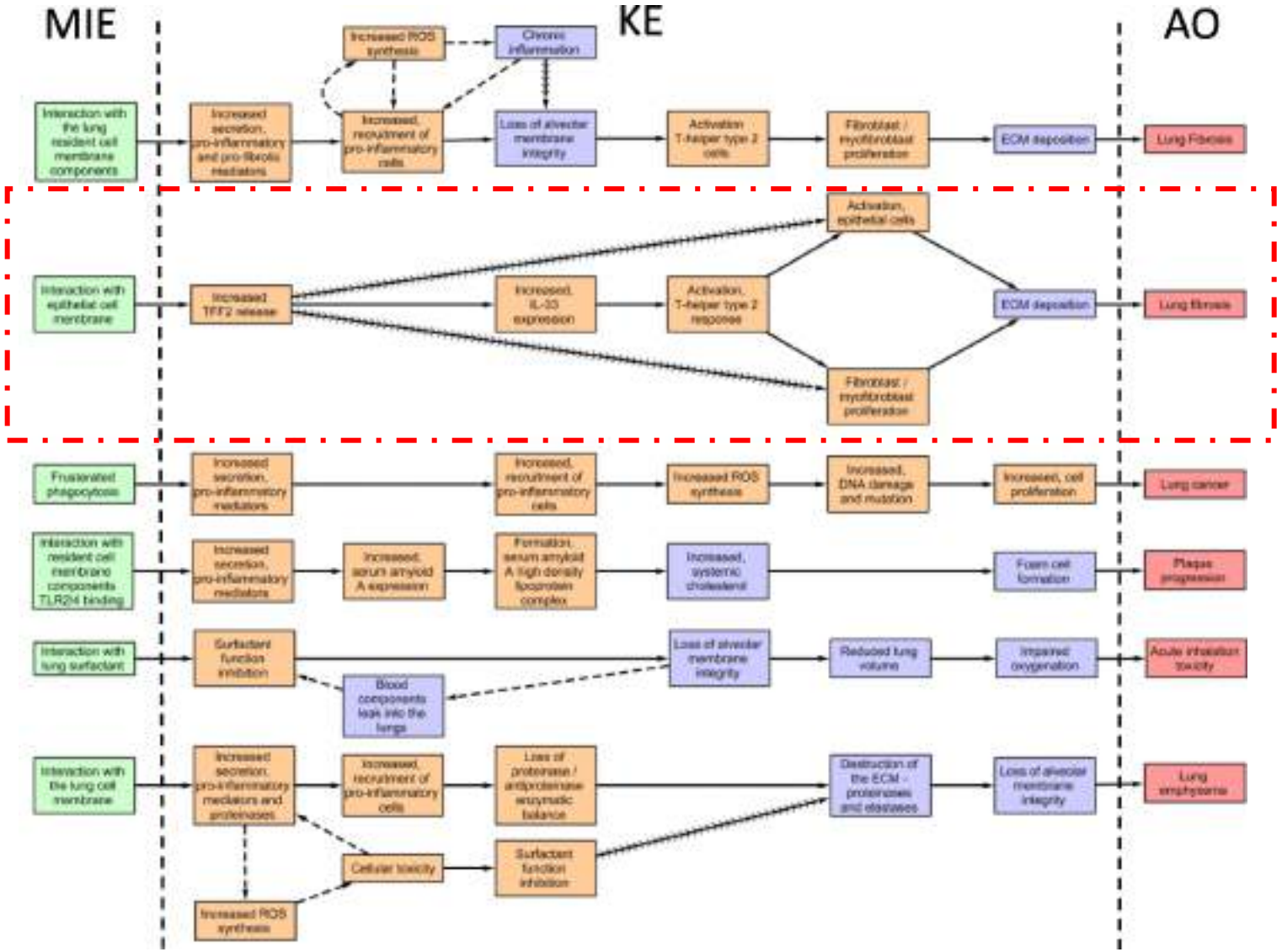
Murine alveolar epithelial cells (AT2) show a unique inflammatory signature for CB/Ptx90



Smart assays for AOP-based mechanism-aware toxicity assessment

Henrik Wolff (FIOH)

Tff2-Il33-Th2 Fibrosis-AOP



Halappanavar et al., P&FT, 2020

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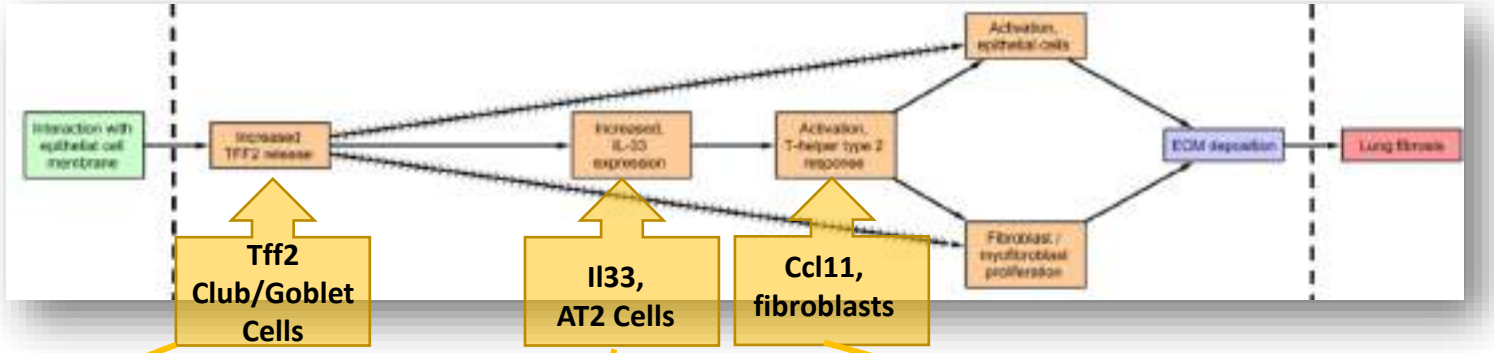
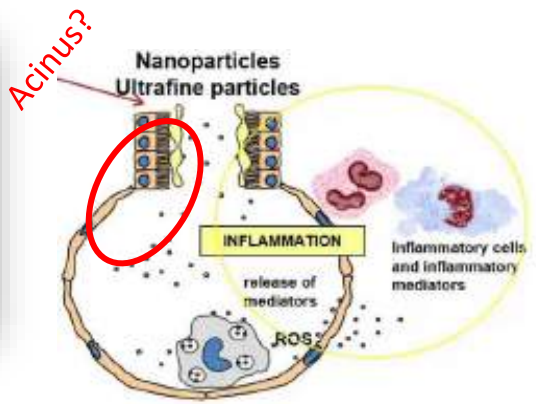
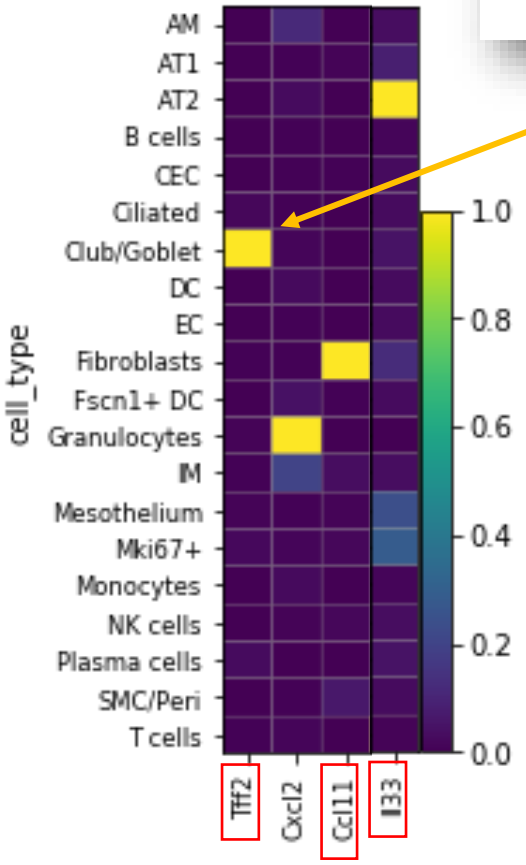




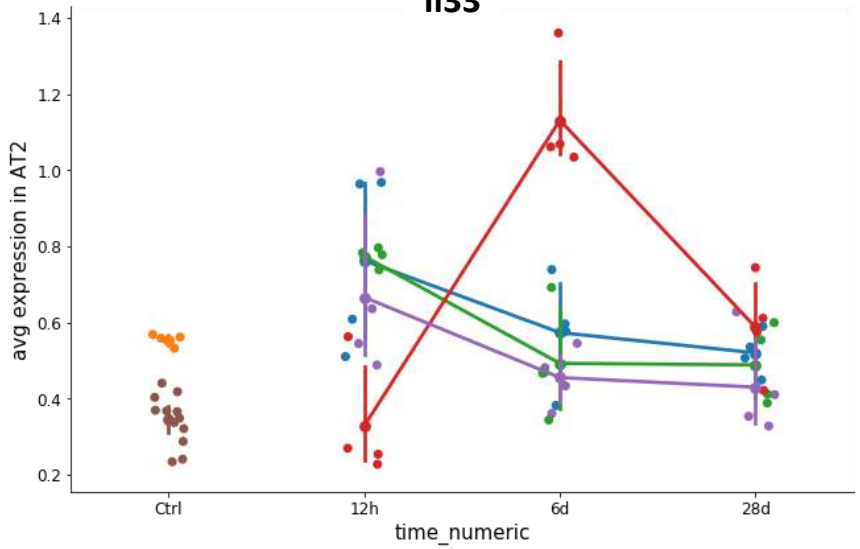
Tff2 Club/Goblet Cells

Il33, AT2 Cells

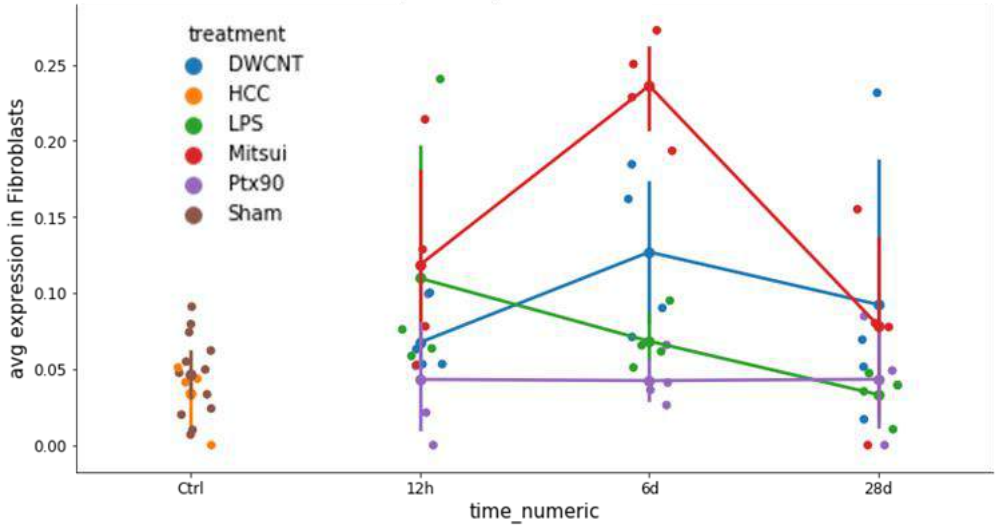
Ccl11, fibroblasts



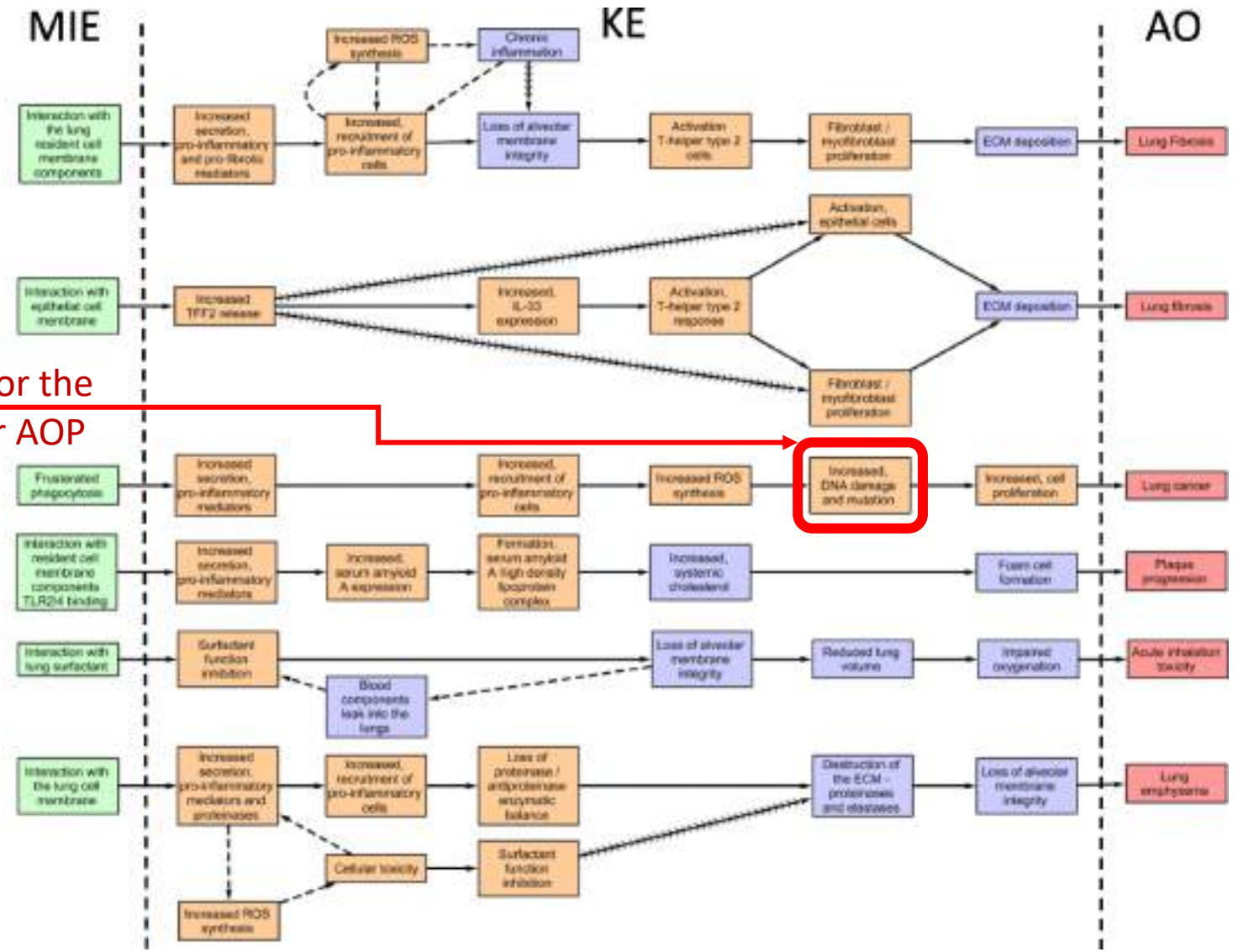
Il33



Ccl11/Eotaxin1



Smart assays for AOP-based mechanism-aware toxicity assessment



Halappanavar et al., P&FT, 2020

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Smart assays for AOP-based mechanism-aware toxicity assessment

Rapid (one week) *in vitro* mutation detection screen

Murine or human benign lung epithelial cells were treated with NMs and environmental carcinogens for 8 hours, and cells where continuing cultured for 7 days, before analysis by RNAseq.

Georgios Stathopoulos

Molecular Lung Carcinogenesis

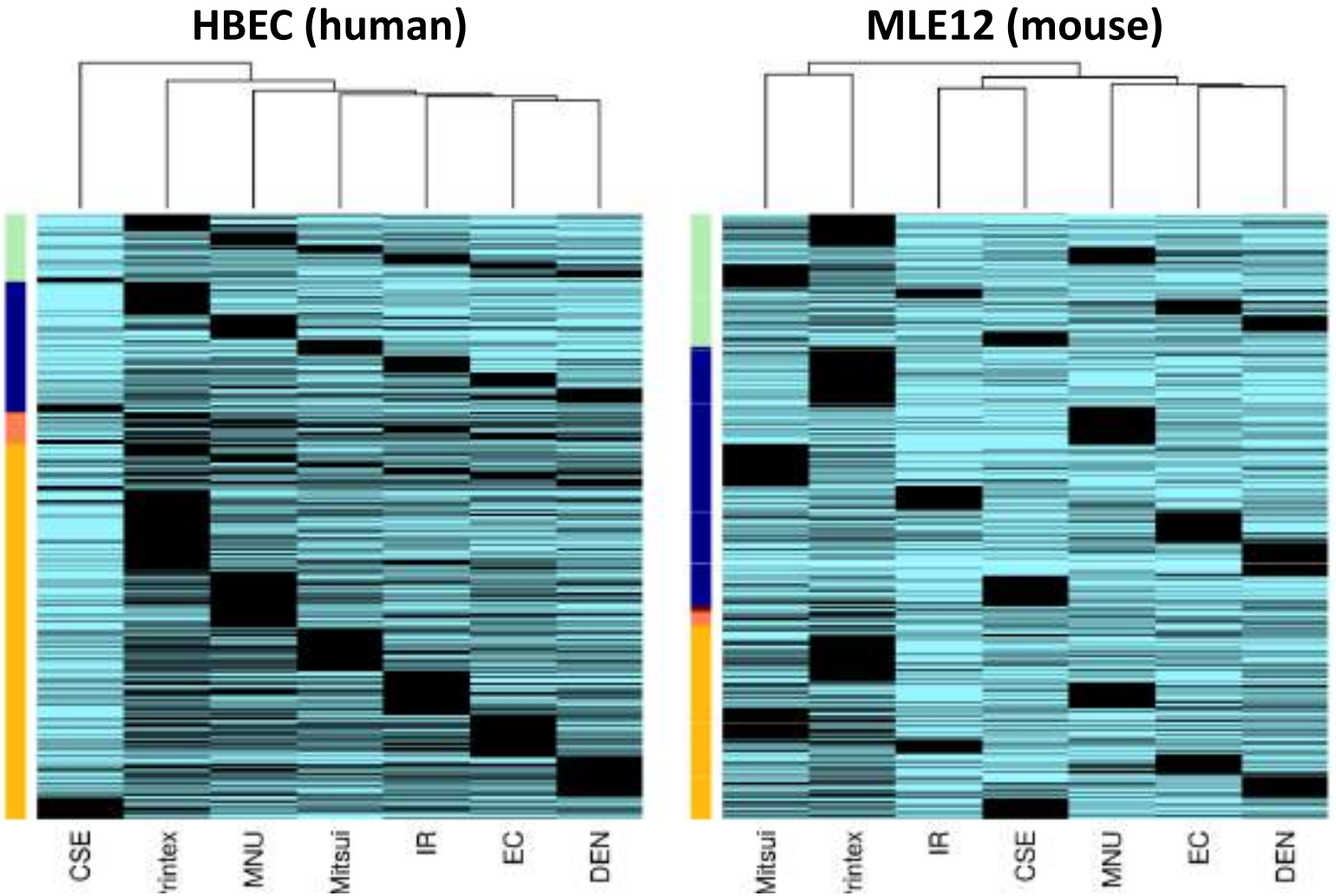
https://www.helmholtz-muenchen.de/ilbd/research/ilbdcpc-research-groups/molecular-lung-carcinogenesis-stathopoulos-lab/main-projects/index.html

Sabine Behrend, Georgia Giotopoulou; Anne-Sophie Lamort; Mario Pepe

in cooperation with Carola Voss, Yaobo Ding, Tobias Stoeger, Otmar Schmid

Smart assay: *in vitro* mutation detection screen

Clustering of DNA polymorphisms detected in mouse and human lung epithelial cells

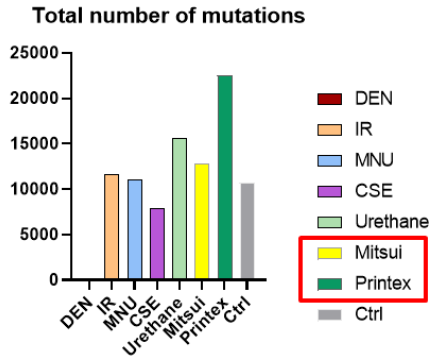


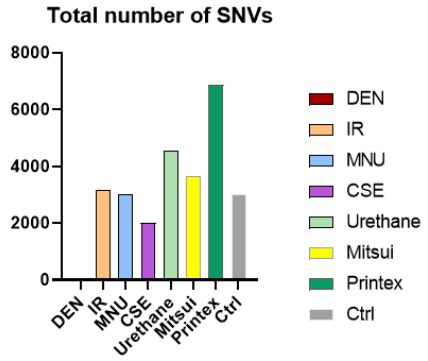
RNA sequencing of human (16HBEo) and mouse (MLE12) lung epithelial cells 7d after a 7h NM exposure with CNP/Ptx90 and CNT/Mitsui7, and several lung carcinogens.
Cells cultured in 6 well plates (DMEM 10%FBS 1%P/S) treated for 7 hours with the doses for IC50, (1/3 IC50 and 3x IC50) daily changed the medium, cultured for 7 days.

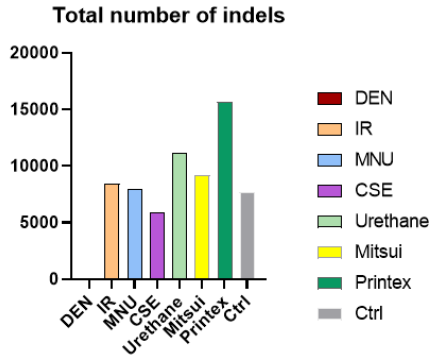
CSE	Cigarette smoke extract
IR	irradiation, 4 gray)
EC	Ethylene carbonate
MNU	Methyl nitrosourea
DEN	Diethylnitrosoamine

Smart assay: *in vitro* mutation detection screen

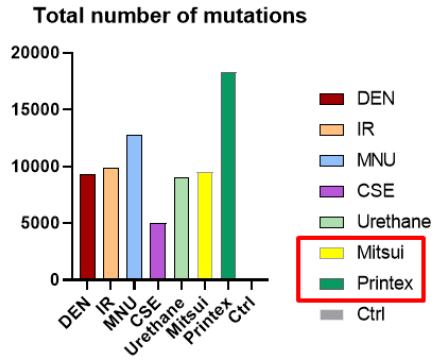
MLE12

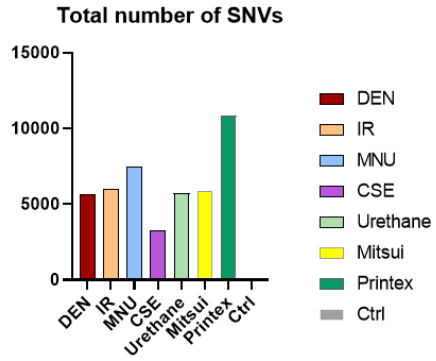


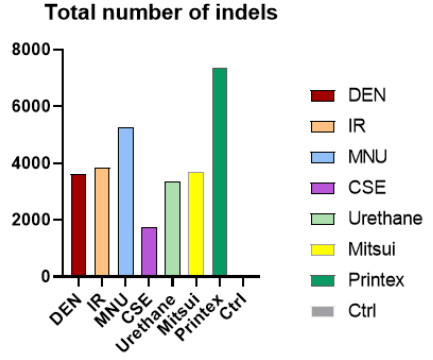




HBEC







➔ **Very promising *in vitro* approach (Smart Assay) for testing the mutagenicity of new materials**

Smart assays for AOP-based mechanism-aware toxicity assessment

List of Smart Assays under development

no.	Smart tests	Lead	AOP
1	**Surfactometer**	NRCWE	Acute Inhalation Toxicity
2	**AM cell irritation (Tox, LysoTr, MM**	UL/HMGU	Chronic Inflammation/Fibrosis/Cancer
3	**AEC irritation (Tox)**	HMGU	Chronic Inflammation/Fibrosis/Cancer
4	**AEC cytokine release**	UL/HMGU	Chronic Inflammation/Fibrosis/Cancer
5	**AM cytotoxicity**	UL/HMGU	Chronic Inflammation/Fibrosis/Cancer
6	**AM frustrated phagocytosis**	UL	Chronic Inflammation/Fibrosis/Cancer
7	**Fibrobl. Acute Phase/ Saa3**	HMGU/NRCWE	Acute Phase Reaction /CVD
8	**PS/NMs aggregate formation**	IJS	TiO2-induced CVD/atherosclerosis
9	**TF/F3 quantification (apical/basal)**	IJS/HMGU	TiO2-induced CVD/atherosclerosis
10	**EC activation in AEC/EC coculture**	IJS/HMGU	TiO2-induced CVD/atherosclerosis
12	**cell(?) Th2 cytokine release**	HMGU/FIOH	Eosinophilic Chronic Inflammation (HARN Injury)
13	**AEC IL33/TFF2**	HMGU/FIOH	Eosinophilic Chronic Inflammation (HARN Injury)
15	**Multi-parameter phys-chem testing**	UCD/HMGU	Acute inflammation (neutrophil/eosinophilic)
16	**coronomics adsorption**	UCD/HMGU	AOP grouping (neutophil/eosinophil)

- **finalized**
- **in progress**
- **problematic**

Thank you for your attention!

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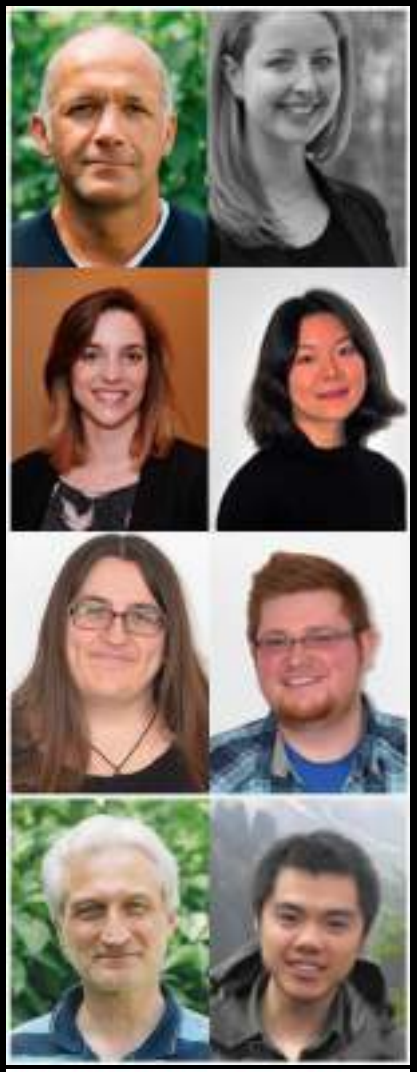

HelmholtzZentrum münchen
German Research Center for Environmental Health

…and great thanks to the
entire SmartNanoTox team
with our colleagues and friends in:
Denmark, Sweden, Norway, Finland, Slovenia,
France, Ireland, UK, and Canada.