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Human health risk assessment of nanomaterials: adverse outcome pathways and key events for the design and development of in vitro predictive tools

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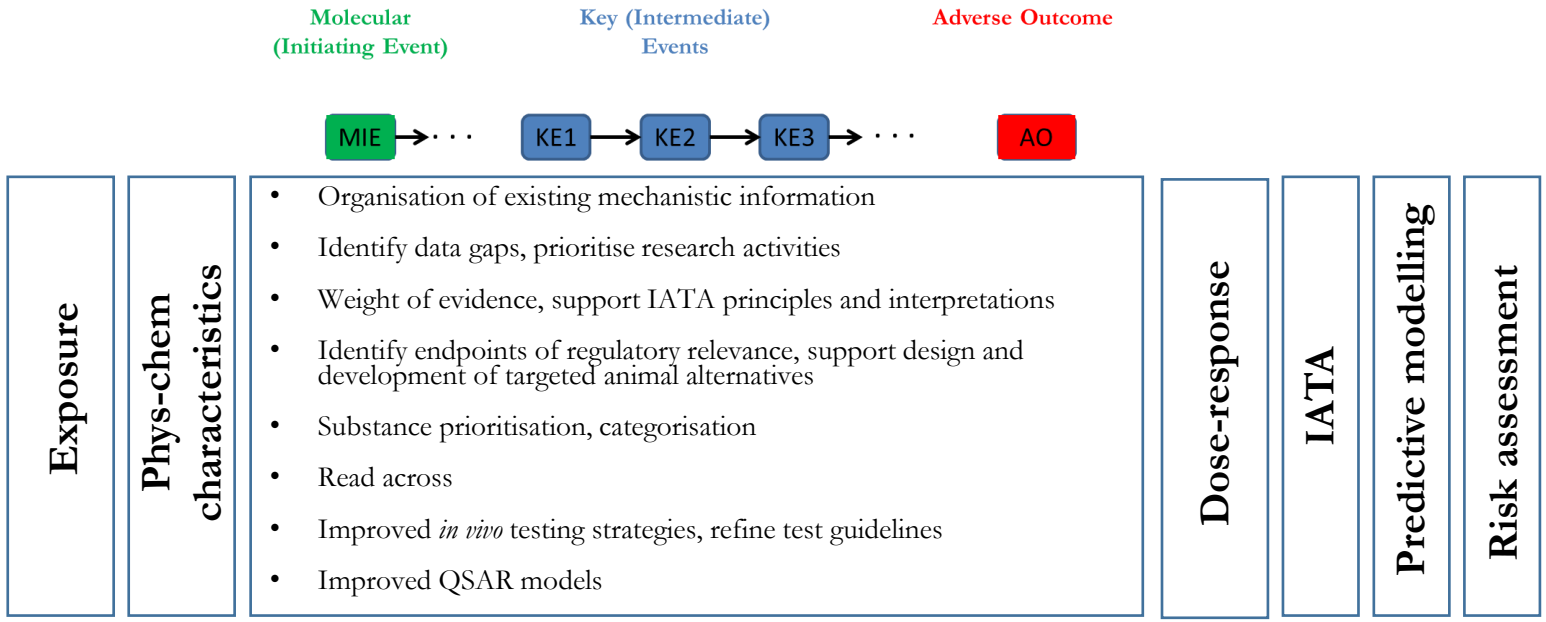
Applications of AOPs for human health risk assessment of nanomaterials



Qualitative, semi-quantitative

Quantitative

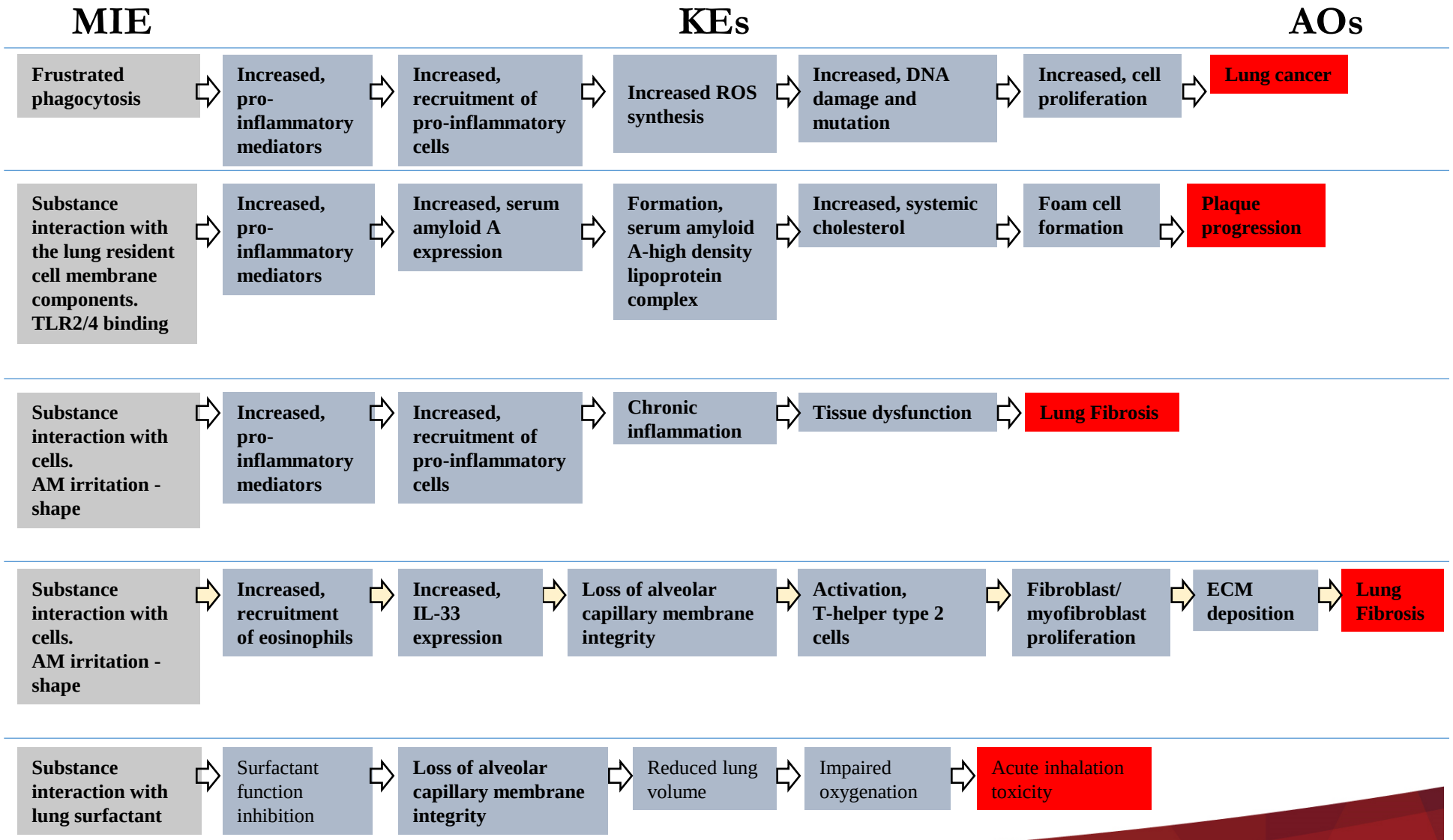
Risk indicators

Halappanavar and Vogel et al., *Under review*

Organisation of mechanistic information

Nano-relevant AOPs currently under development at HC and by SmartNanoTox

Sabina Halappanavar; Laurent Gate; Jorid Birkelund Sørli (JBS); Tobias Stöger; Wolff Henrik; Carole Seidel; Ulla Vogel

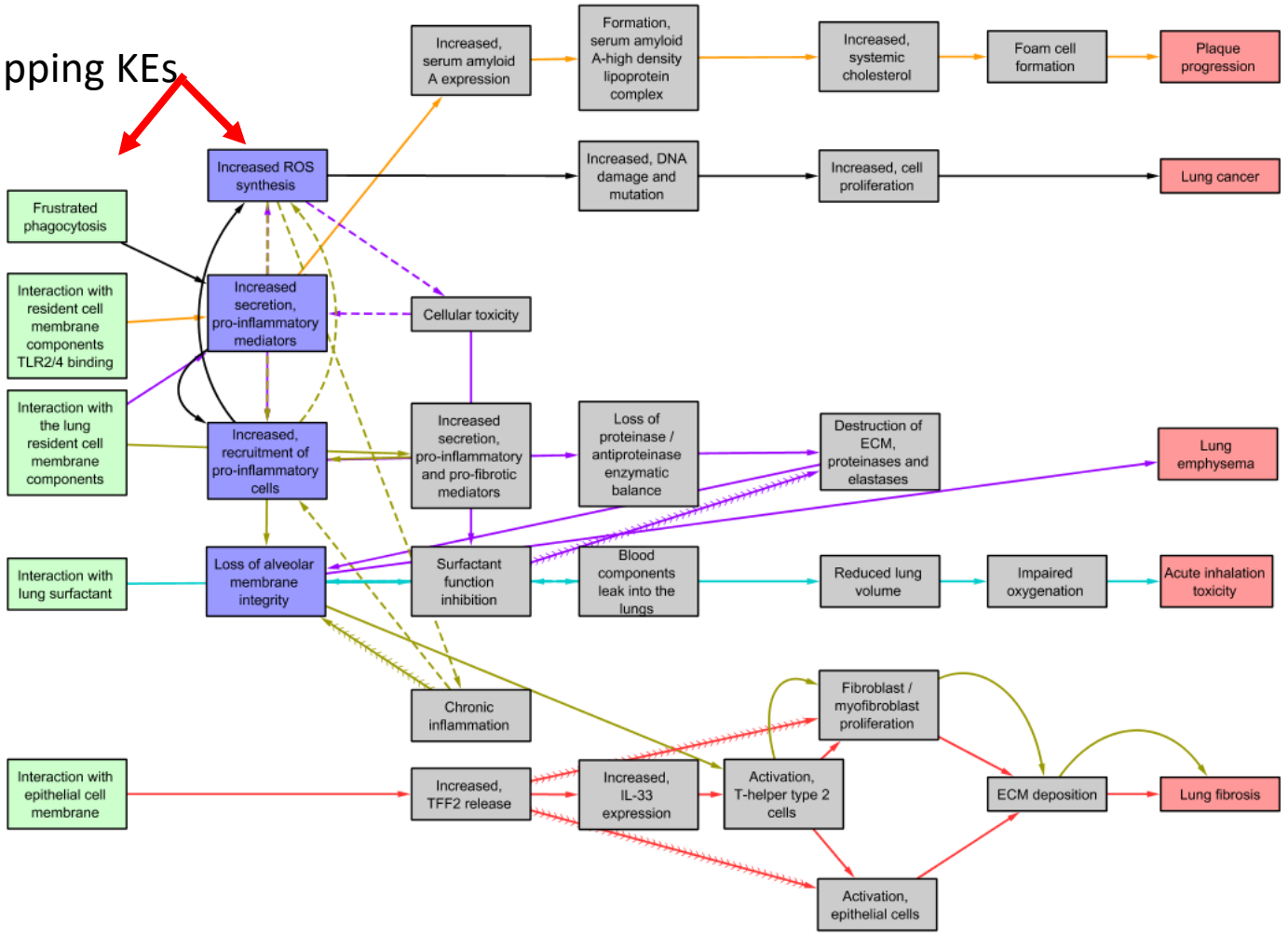


Halappanavar and Vogel et al., *Part Fibre Toxicol*. 2020;17(1):16. Published 2020 May 25.

Identification of key events for targeted in vitro endpoints/assays development

Network of Nano-relevant AOPs of importance to inhalation exposure

Node/overlapping KEs



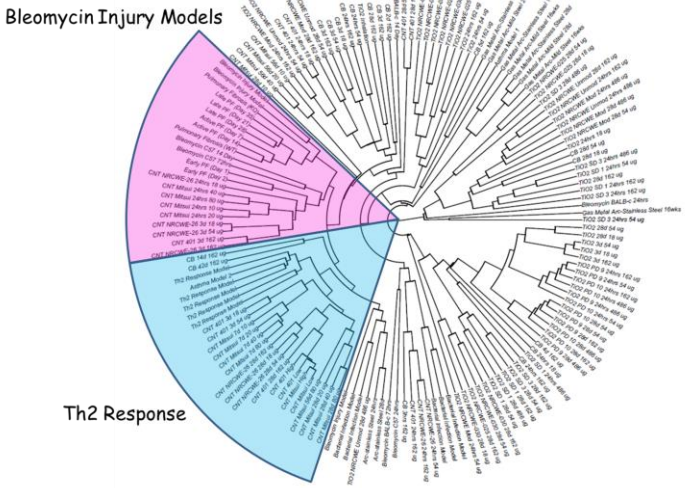
Halappanavar and Vogel et al., *Part Fibre Toxicol*. 2020;17(1):16. Published 2020 May 25.

Halappanavar and Vogel et al., *Under review*

Overlapping KEs	**In vivo inhalation toxicity assays (OECD), (2018) ENV/JM/MONO(2009)28/REV1**	Potential animal alternatives Non-validated	
		Endpoints	**Systems/models**
MIE - Substance interaction with the resident cell membrane components.		Expression changes in alarmins, lysosomal enzymes, receptor pathway activation, cellular uptake, lysosomal destabilisation assays NM-protein, NM-lipid interactions, surfactant functionality assay Resident immune cell mobilisation - histology **Ex Vivo**	**Statistically determined gene signature targeting multiple KEs** **In vitro** EpiAlveolar constructs, co-culture models, single cell models **Ex vivo** Precision cut lung slice model
KE - Increased, proinflammatory and profibrotic mediators **KE -** Increased, recruitment of proinflammatory cells	**Bronchoalveolar lavage fluid parameters –** -Lactate dehydrogenase -Total protein or albumin -Total leukocyte count, -Absolute and differential cell counts **Histology**	Expression changes in proinflammatory and profibortic mediators	
KE - Loss of alveolar capillary membrane integrity	**Bronchoalveolar lavage fluid parameters –** -Lactate dehydrogenase -Total protein or albumin **Lung burden – persistence of NM**	Transepithelial electrical resistance Cell death/cytotoxicity	**In vitro** EpiAlveolar constructs, co-culture models, single cell models
AE/KE – ROS synthesis		ROS levels	

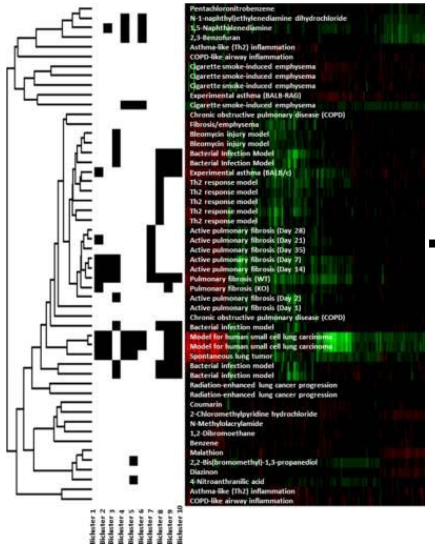
Health Canada approach
Bioinformatics – AOPs – predictive models

Williams & Halappanavar (2015). Beilstein J Nanotechnol. 6:2438-2448;
Nikota et al., (2016). PFT. 11;13(1):25; Labib et al., (2016). PFT. 13, 15;
Nikota et al., (2017). PFT. 14, 37; Rahman et al., (2020). Small. 2020;e2000272.

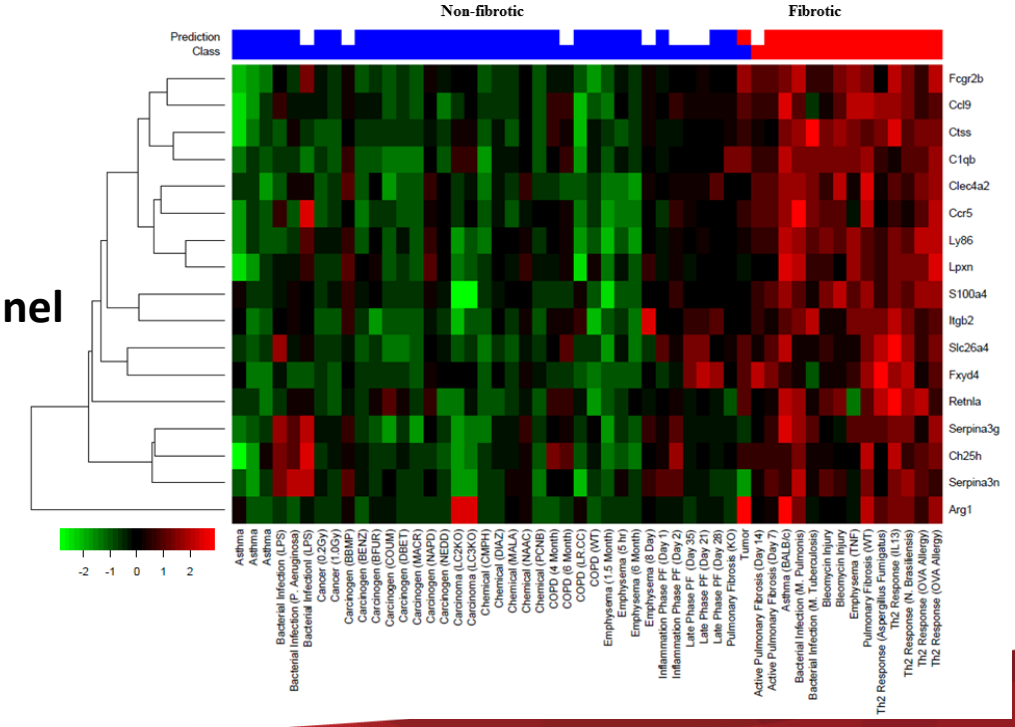


Substance interaction with the resident cell membrane components. → Increased, proinflammatory and profibrotic mediators → Increased, recruitment of proinflammatory cells → Loss of alveolar capillary membrane integrity → T-helper type 2 cells, Activation → Fibroblast/ myofibroblast proliferation → ECM deposition → Lung Fibrosis

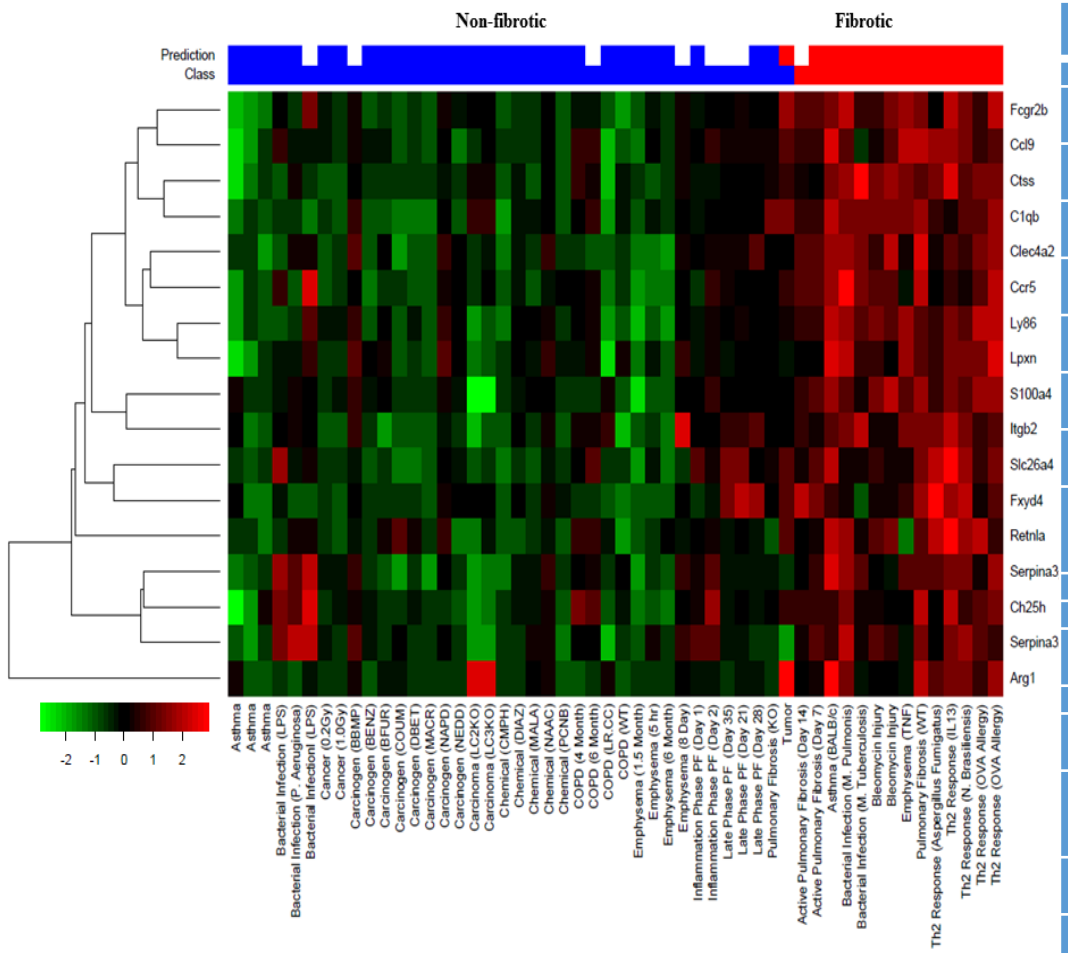
AOP173 Lung fibrosis



17-gene panel



Predictive tools for nanomaterial-induced lung fibrosis:
Establishment of a 17-gene panel (PFS17)



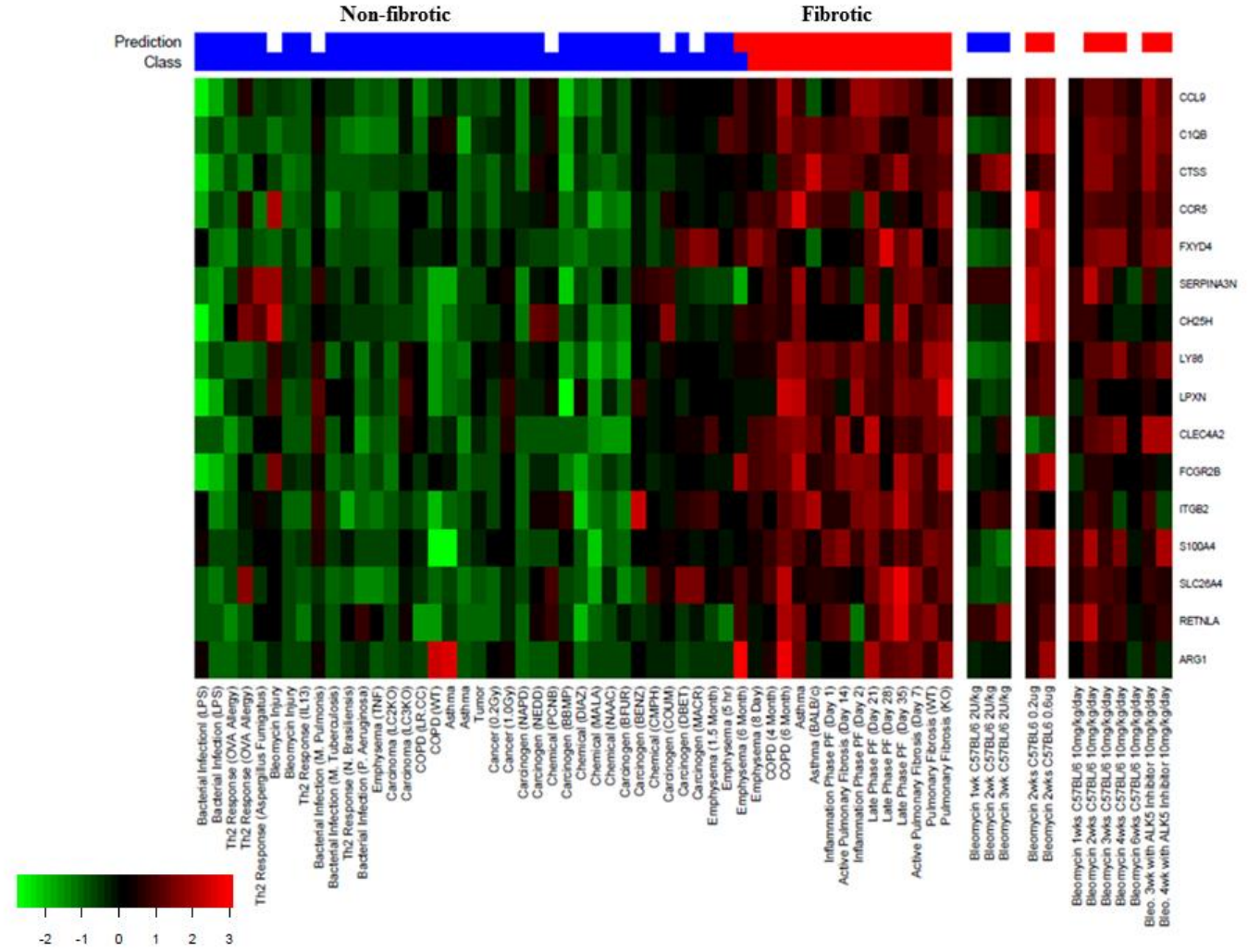
Training set

Gene Symbol	Accession no.	Gene Name
Arg1	NM_007482.3	Mus musculus arginase 1, liver (Arg1), mRNA
C1qb	NM_009777.2	Mus musculus complement component 1, q subcomponent, beta polypeptide (C1qb), mRNA
Ccl9	NM_011338.2	Mus musculus chemokine (C-C motif) ligand 9 (Ccl9), mRNA
Ccr5	NM_009917.5	Mus musculus chemokine (C-C motif) receptor 5 (Ccr5), mRNA
Ch25h	NM_009890.1	Mus musculus cholesterol 25-hydroxylase (Ch25h), mRNA
Clec4a2	NM_001170333.1	Mus musculus C-type lectin domain family 4, member a2 (Clec4a2), transcript variant 1, mRNA
Ctss	NM_021281.3	Mus musculus cathepsin S (Ctss), transcript variant 2, mRNA
Fcgr2b	NM_001077189.1	Mus musculus Fc receptor, IgG, low affinity IIb (Fcgr2b), transcript variant 1, mRNA
Fxyd4	NM_001173372.1	Mus musculus FXYD domain-containing ion transport regulator 4 (Fxyd4), transcript variant 2, mRNA
Itgb2	NM_008404.4	Mus musculus integrin beta 2 (Itgb2), mRNA
Lpxn	NM_134152.3	Mus musculus leupaxin (Lpxn), mRNA
Ly86	NM_010745.2	Mus musculus lymphocyte antigen 86 (Ly86), mRNA
Retnla	NM_020509.3	Mus musculus resistin like alpha (Retnla), mRNA
S100a4	NM_011311.2	Mus musculus S100 calcium binding protein A4 (S100a4), mRNA
Serpina 3g	NM_009251.2	Mus musculus serine (or cysteine) peptidase inhibitor, clade A, member 3G (Serpina3g), transcript variant 1, mRNA
Serpina 3n	NM_009252.2	Mus musculus serine (or cysteine) peptidase inhibitor, clade A, member 3N (Serpina3n), mRNA
Slc26a4	NM_011867.4	Mus musculus solute carrier family 26, member 4 (Slc26a4), mRNA

Rahman L, Williams A, Gelda K, et al. [published online ahead of print, 2020 Apr 29]. *Small*. 2020;e2000272.

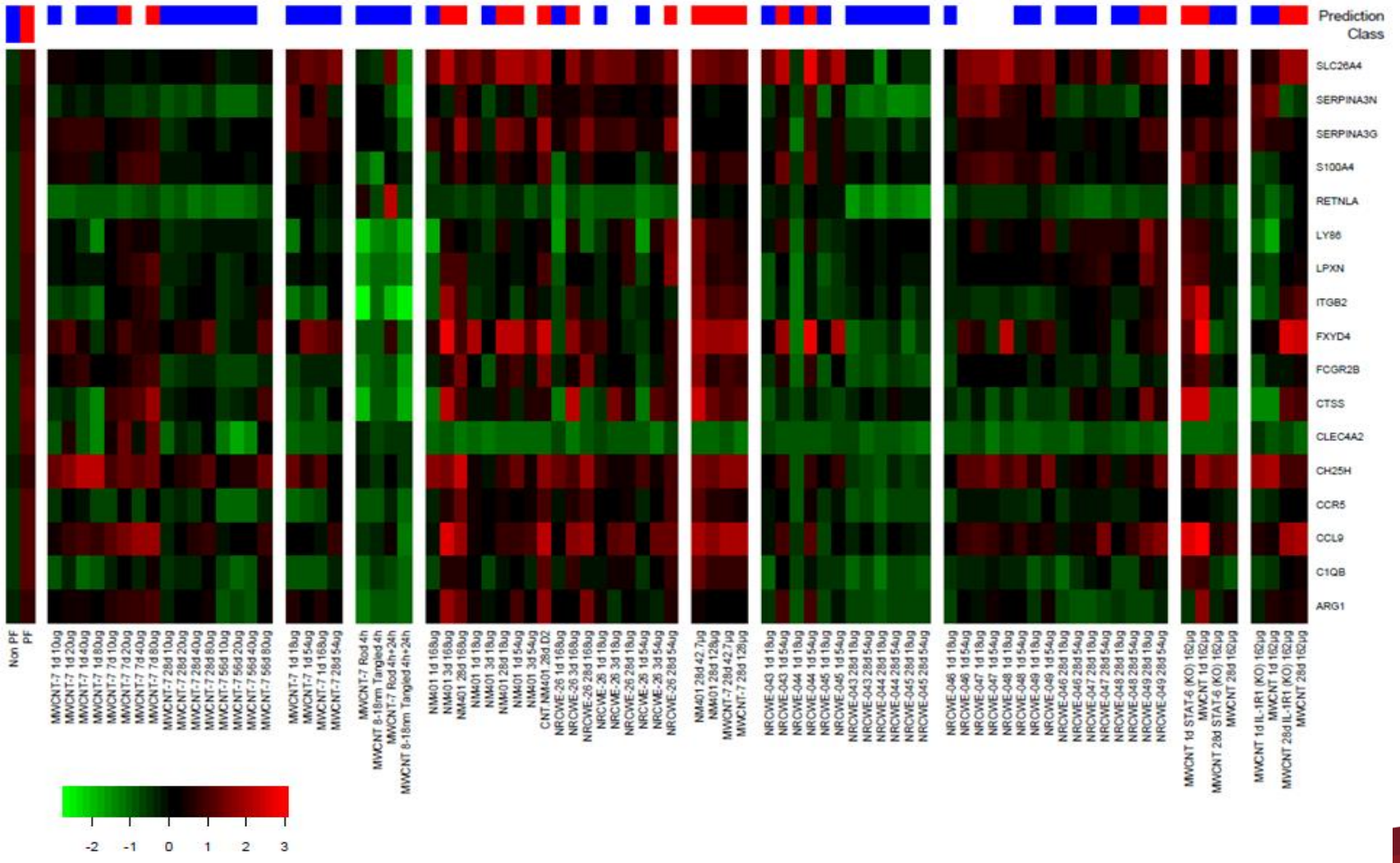
Predictive tools for nanomaterial-induced lung fibrosis:

PFS17 validation – bleomycin studies



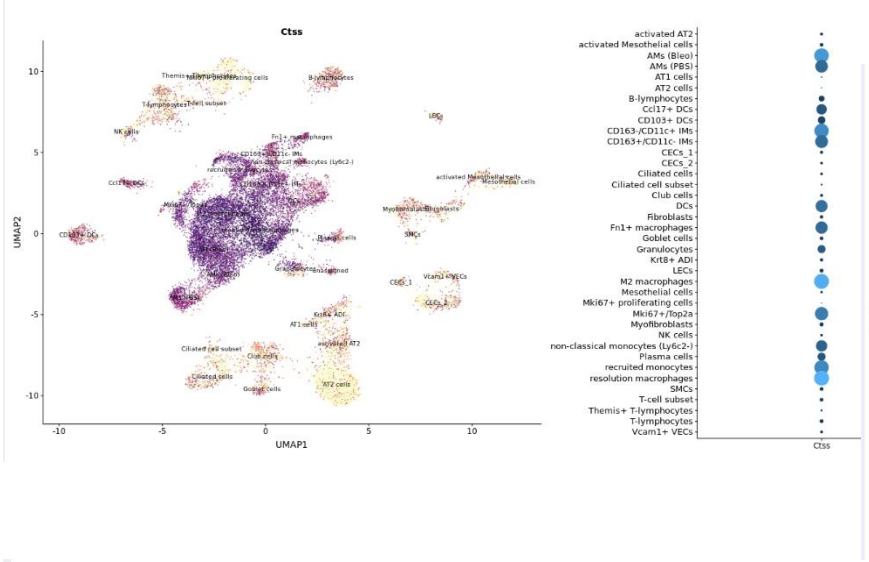
Predictive tools for nanomaterial-induced lung fibrosis:

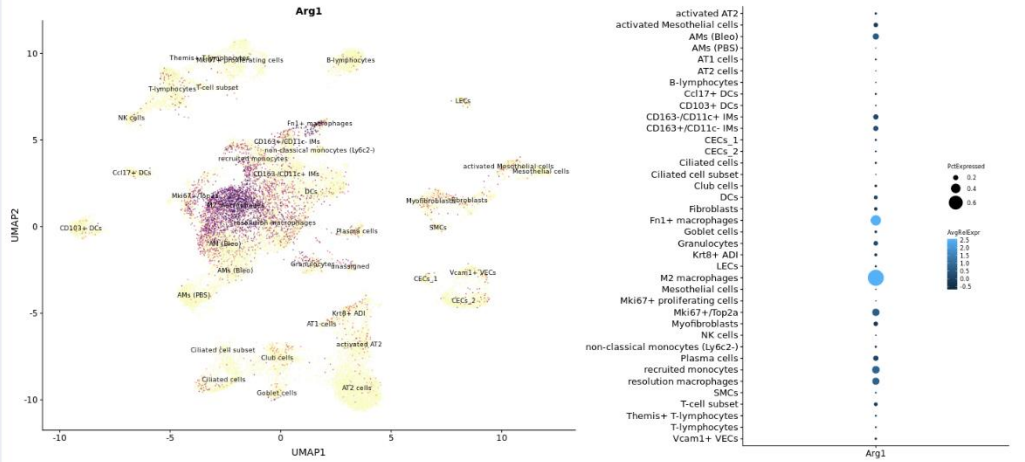
PFS17 validation – Nano studies

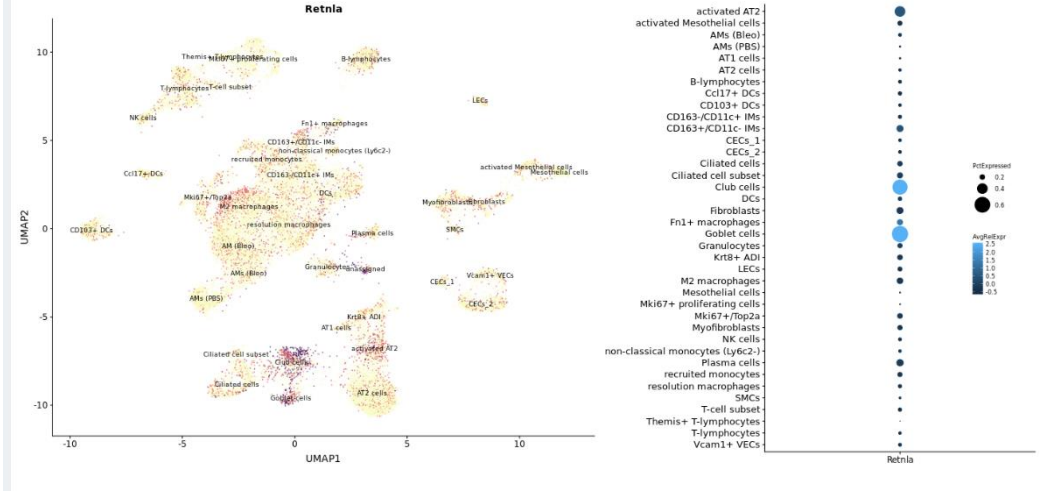


Expression of PFS17 in bleomycin exposed lung cells – multiple cells involvement

Courtesy of Tobias Stoeger







Longitudinal single cell transcriptomics reveals Krt8+ alveolar epithelial progenitors in lung regeneration

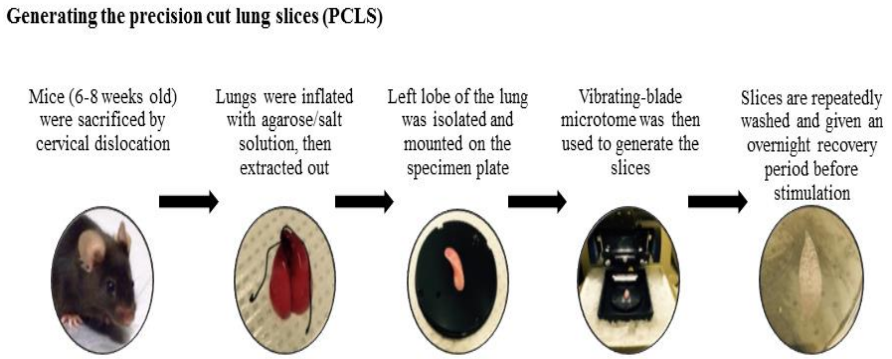
Maximilian Strunz, ……. Tobias Stöger, Mareike Lehmann, Melanie Königshoff, Gerald Burgstaller, Michael O'Reilly, Harold A. Chapman, Fabian J. Theis*, and Herbert B Schiller***doi:** https://doi.org/10.1101/705244https://www.biorxiv.org/content/10.1101/705244v2

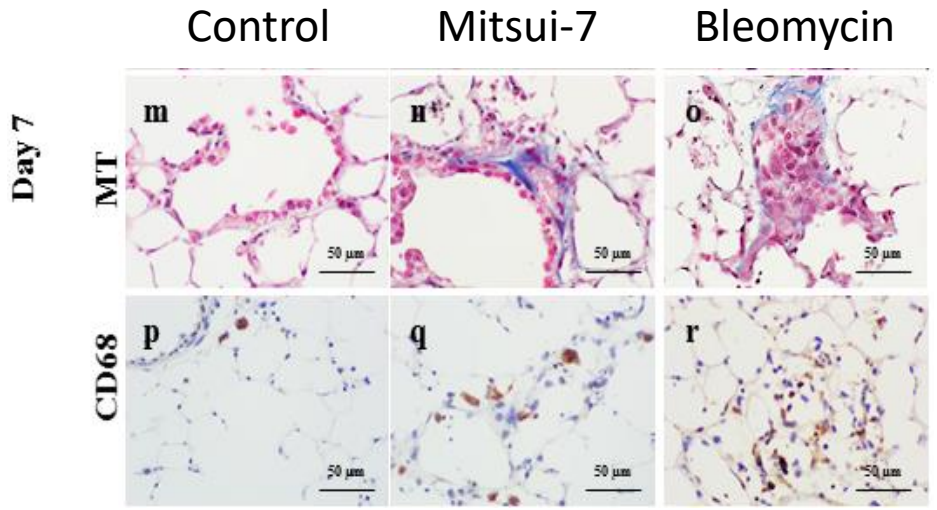
Mouse Lung Injury & Regeneration – Schiller & Theis labs @ Helmholtz Center Munich

http://146.107.176.18:3838/Bleo_webtool_v2/

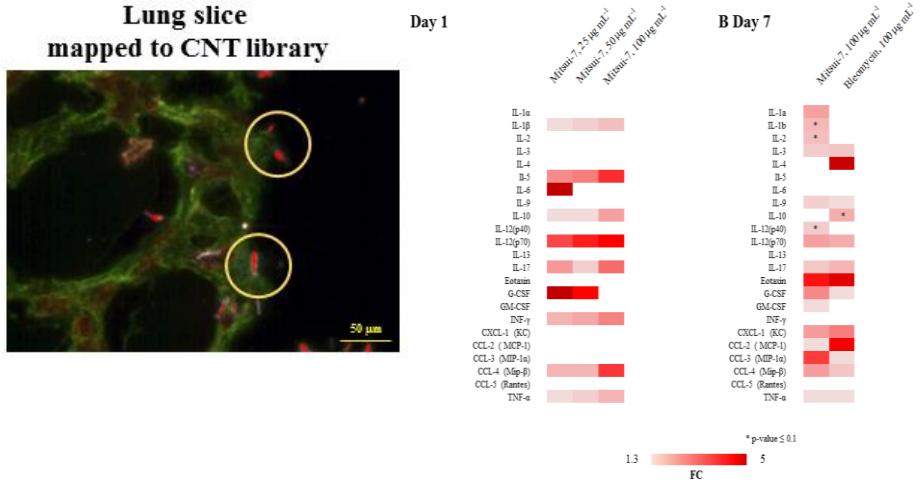
Halappanavar et al., Unpublished data

Ex vivo precision cut lung slice model, PFS17 and AOP173: an animal alternative strategy to assessing lung fibrosis



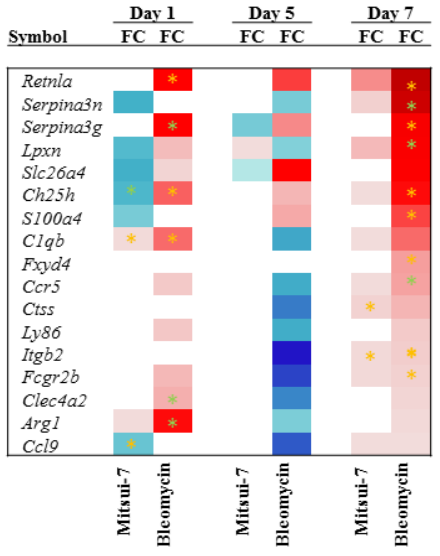


AO – histological evidence



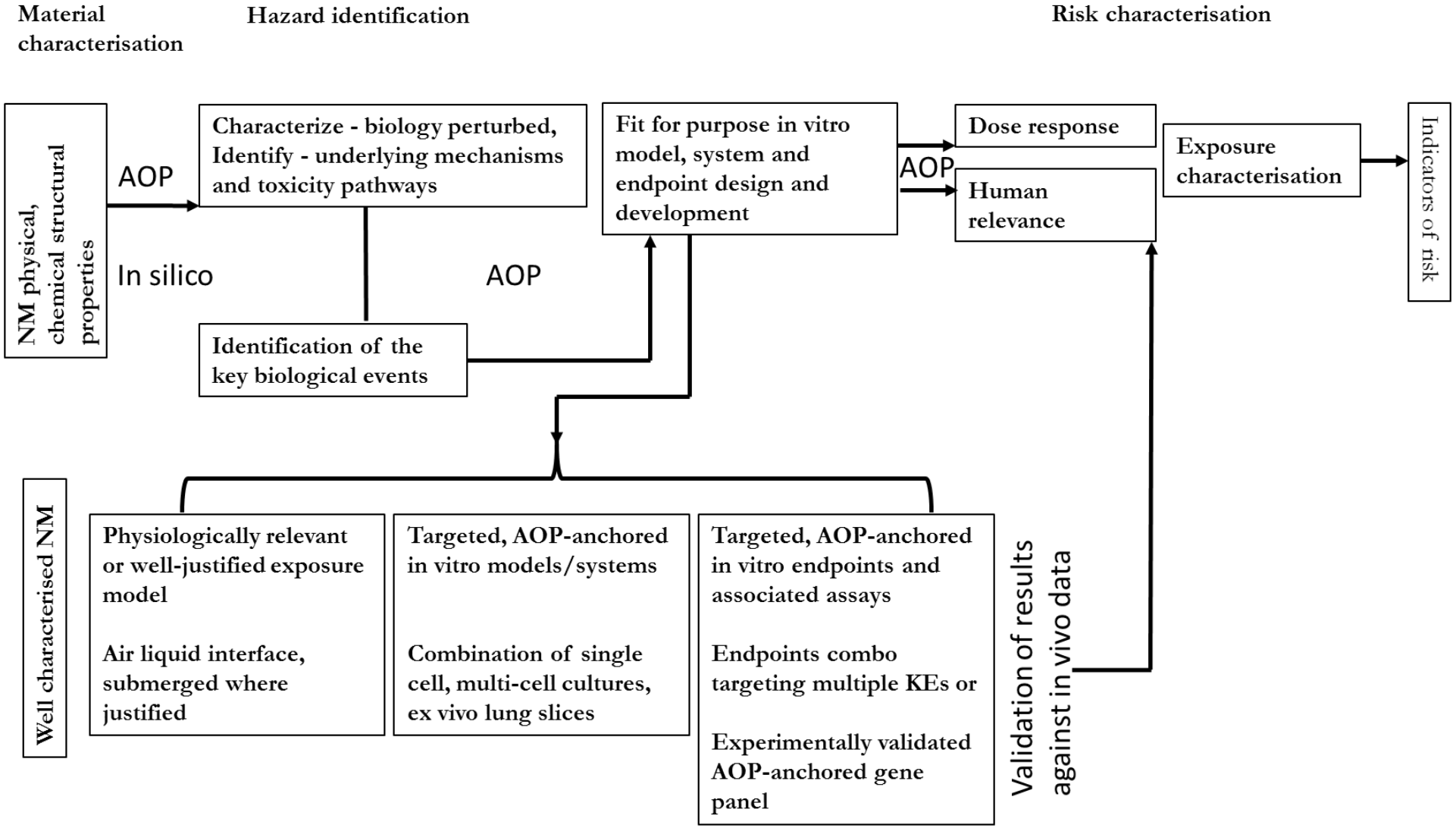
MIE, KE1-KE2

PFS17



KE1-KE6

Conclusions: perspectives



Statistically determine the combination of alternative endpoints/assays with best predictive efficiency

Halappanavar and Vogel et al., *Under review*

Acknowledgements

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Genomics Research and Development Initiative

Chemicals Management Plan

SmartNanoTox
Smart Tools for Gauging Nano Hazards

Ulla Vogel

<u>AOP developers</u>
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Carole Seidel
Vadim Zernovkov

Tobias Stöger