Are we overthinking AOPs?

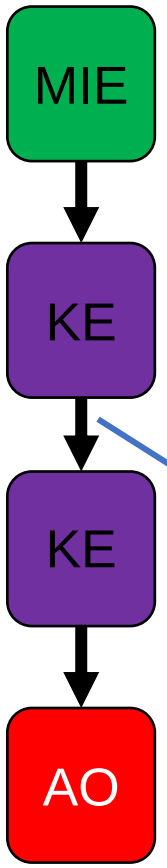
Sabina Halappanavar, Ph.D.
Research Scientist,
Nanotoxicology and Microplastics Toxicology Laboratory,
Health Canada

Adjunct Professor, Department of Biology
University of Ottawa

Ottawa, Canada
April 03, 2024

Adverse Outcome Pathways (AOPs)

- AOPs are modular constructs of knowledge
- They enable collection, organization and presentation of knowledge in a simplistic manner
- Individual AOPs reveal a single mechanism of toxicity



Molecular Initiating Event (MIE) is the initial interaction between toxicants and biomolecules

Key Events (KE) are measurable biological events that are essential for the progression of the pathway

Key Event Relationships (KER) describe the causal relationship between two KEs characterised by:

- Incidence, dose and temporal concordance

AO is an endpoint of regulatory concern

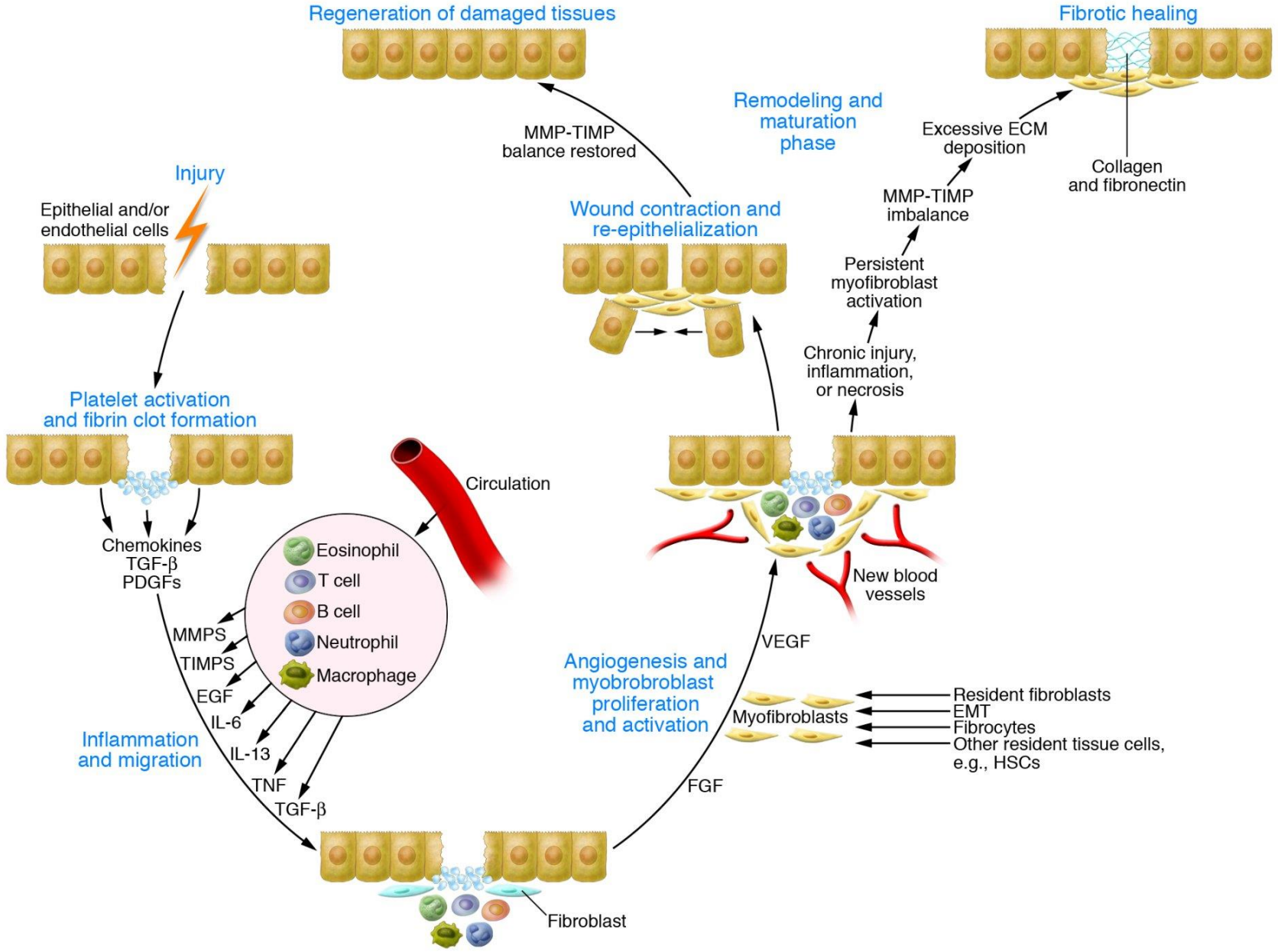
Mechanism of Fibrosis – in Humans and in Animals

Thomas Wynn. Clin Invest. 2007;117(3):524–529.

- Initiation, progression and manifestation of any biological or toxicological event involves mechanisms of its own

The AOPs help with

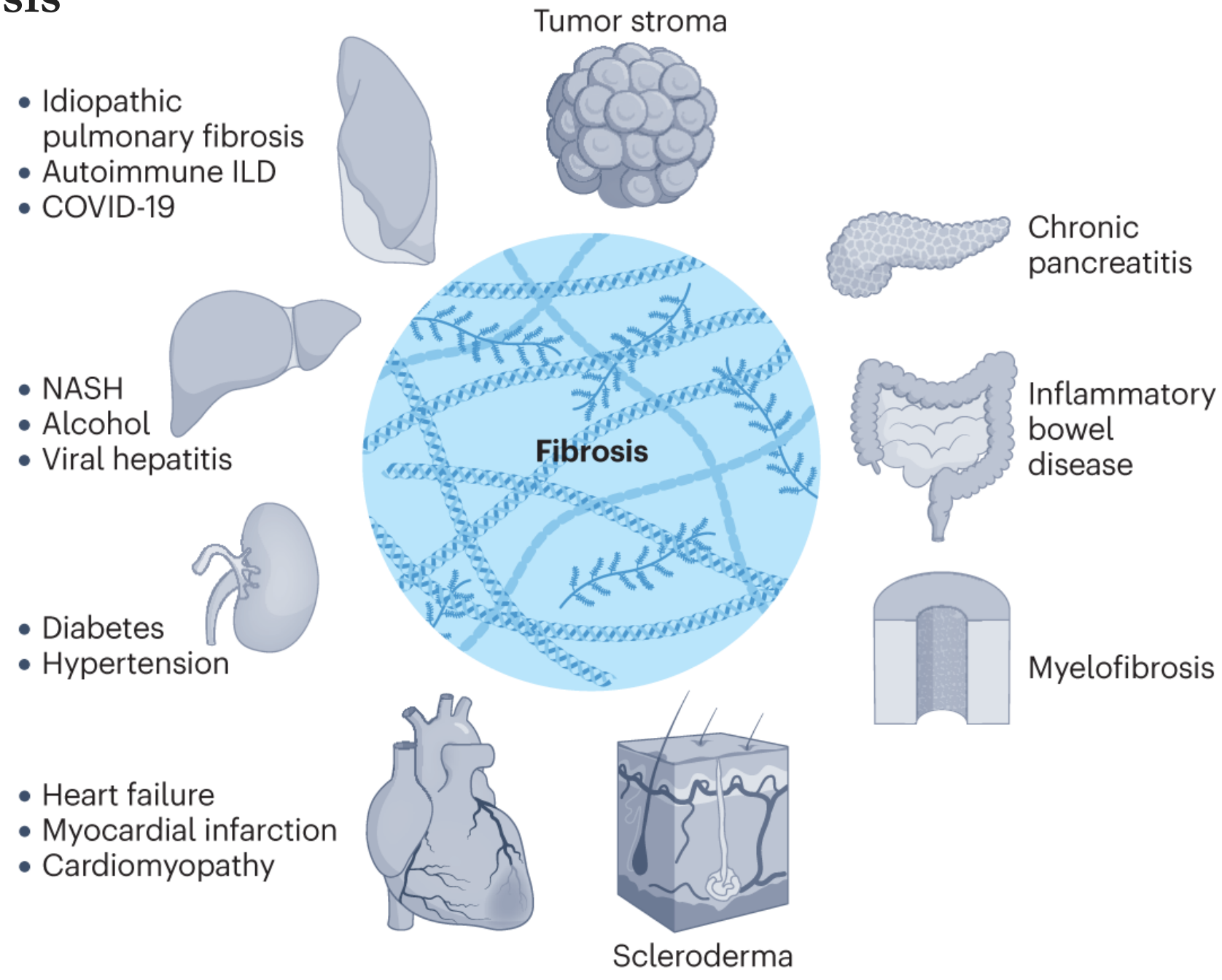
- Finding the most essential events, characterization of the events
- Establishing causality, essentiality, dose-response



Tissular Diversity of Fibrosis

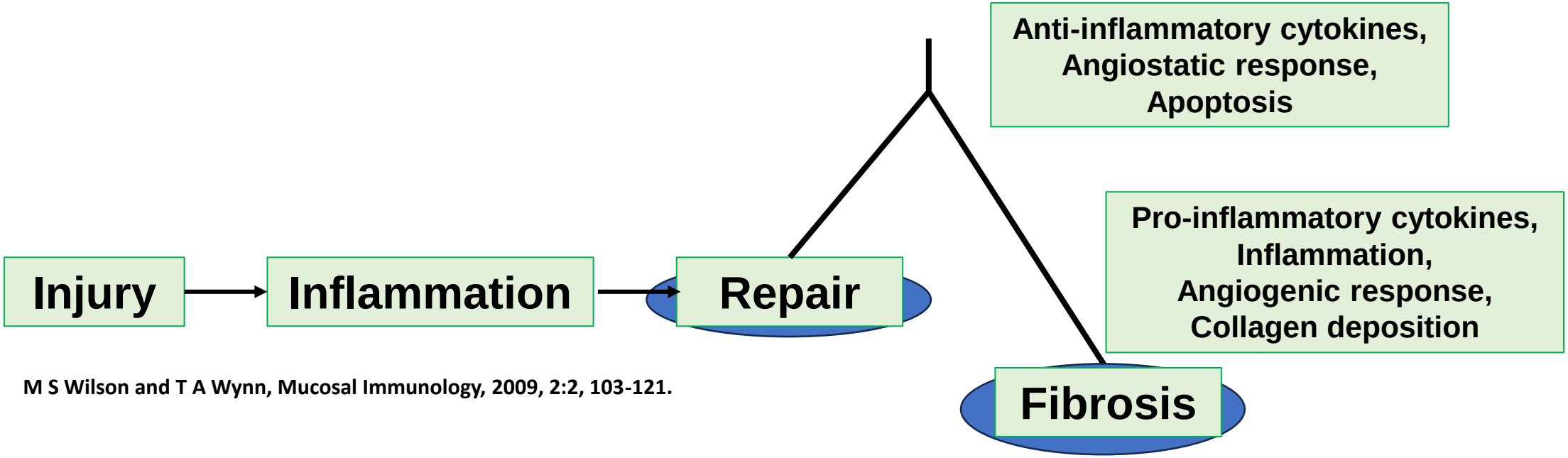
Bhattacharya, et al., *Nat Immunol* 24, 1423–1433 (2023)

Fibrosis is the pathological outcome of an abnormal wound healing process



Mechanism of Fibrosis – in Humans and in Animals

Normal vs Fibrosis

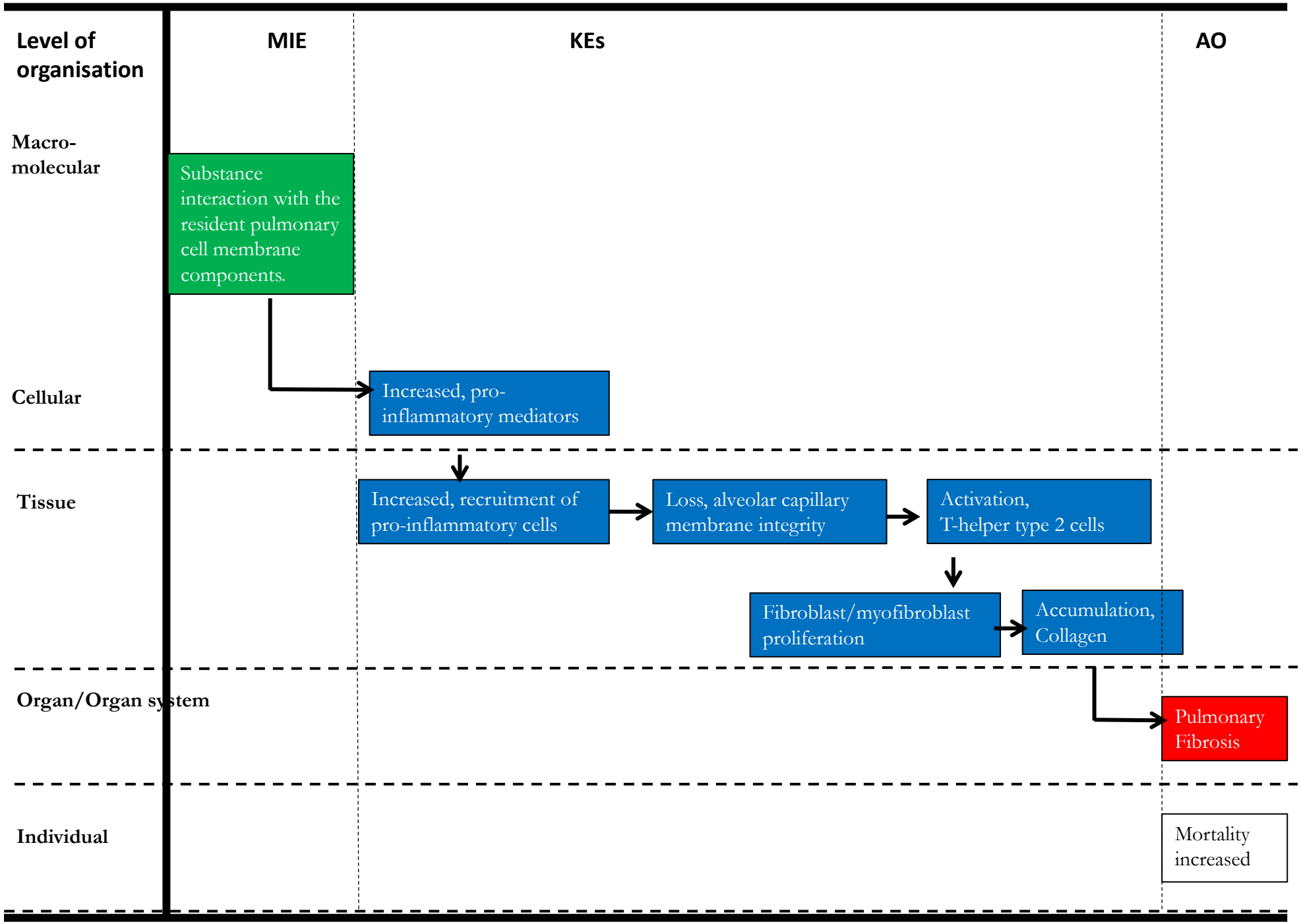


M S Wilson and T A Wynn, Mucosal Immunology, 2009, 2:2, 103-121.

AOP 33 -
previously AOP 173

Halappanavar, S., et al. (2023), "Substance interaction with the pulmonary resident cell membrane components leading to pulmonary fibrosis", *OECD Series on Adverse Outcome Pathways*, No. 33, OECD Publishing, Paris, https://doi.org/10.1787/10372cb8-en

- Lung tissue
- Insoluble, biopersistent substances
- Induce cell injury
- Induce inflammatory response



AOP 33 Opportunities and Shortcomings

Opportunities

- Sorted information and aggregated knowledge
- A mechanism identified and linked to apical endpoints
- Conserved mechanism across species, across tissues
- Visualisation of gaps in knowledge – direct research needs
- Laid foundation for the development of targeted test methods – (in vivo – in vitro translation)

Limitations

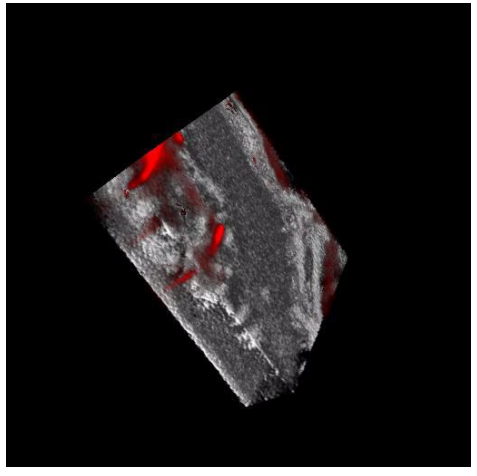
- Non-specific MIE, single MIE not enough
- Temporal relationship not captured but required for fibrosis
- Deals with defense mechanisms – often are compensated

Critical next steps - immediate

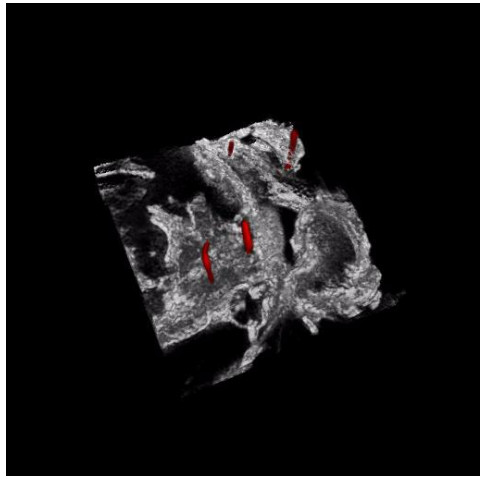
- Identify in vitro surrogates (models, assays, techniques) of in vivo events
- Criteria for validation of AOP-driven assays and techniques

AOP 173/33 for nanomaterials

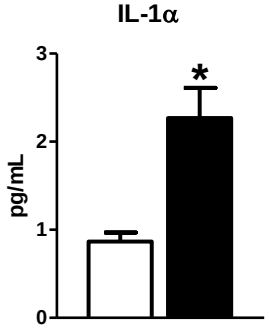
Mitsui-7

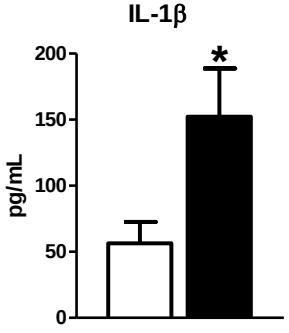


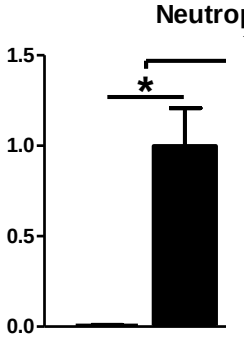
NM-401



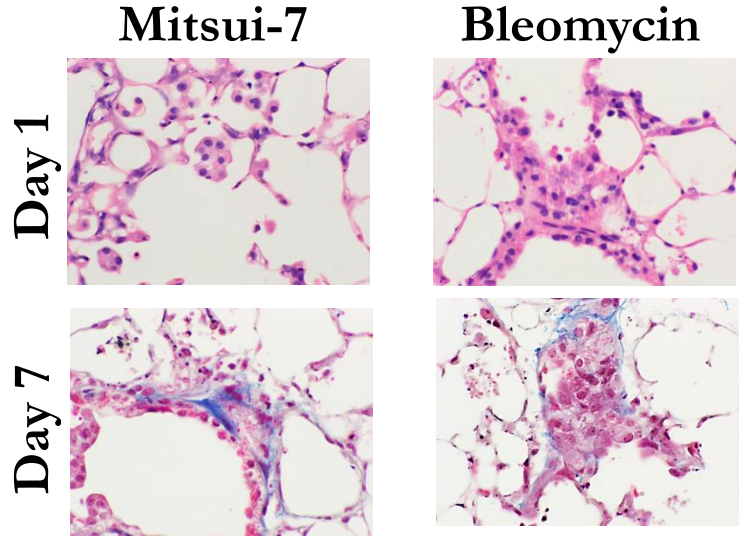
MIE – cellular interaction → Cell/tissue injury → KE - Inflammation





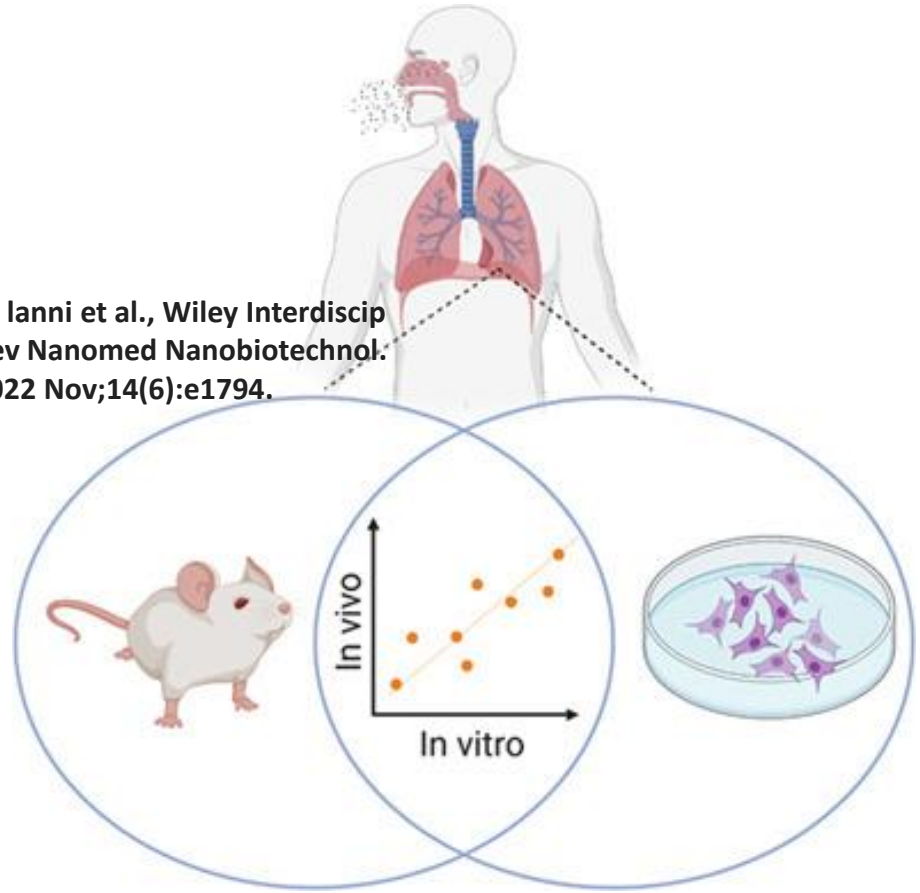


KE/AO – Collagen accumulation



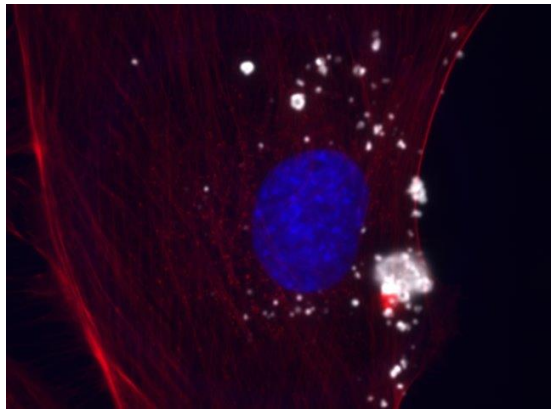
Nikota et al., Part Fibre Toxicol. 2017 Sep 13;14(1):37.
Rahman et al., Small. 2020 Sep;16(36):e2000272.

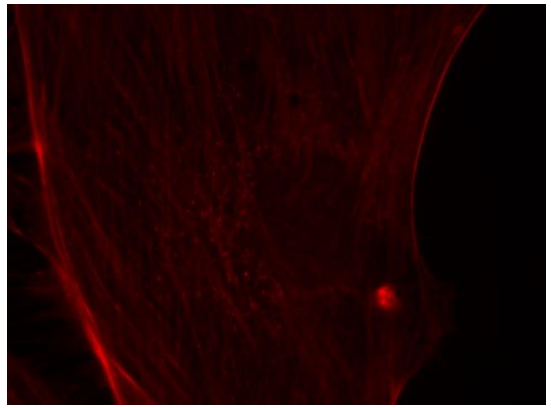
The goal

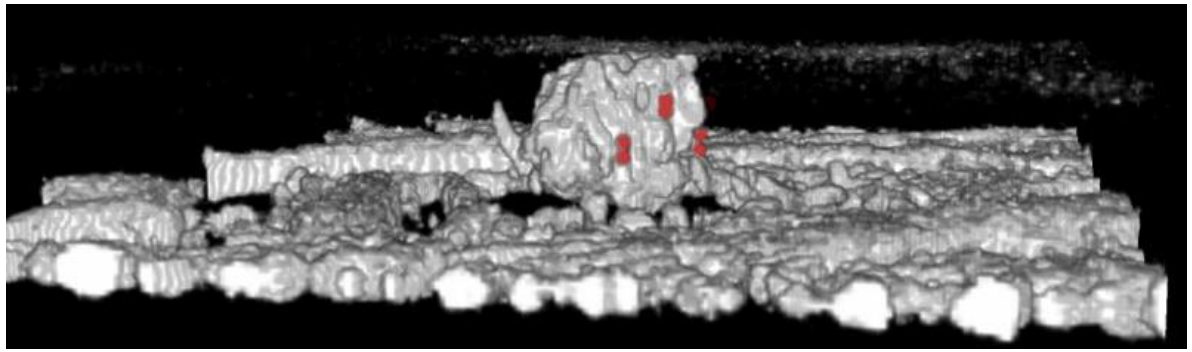


Di Ianni et al., Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2022 Nov;14(6):e1794.

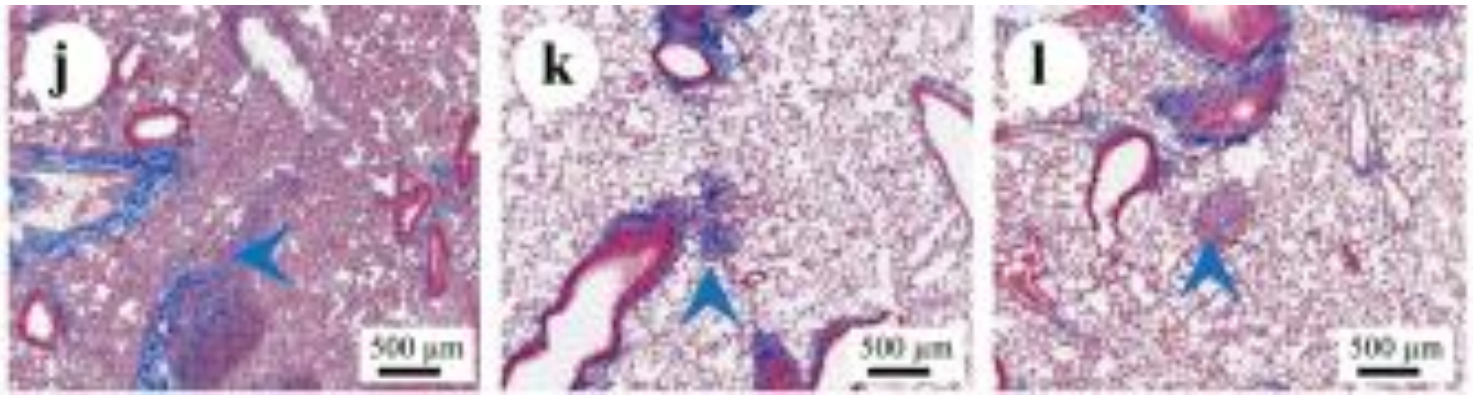
In vitro – cellular interaction





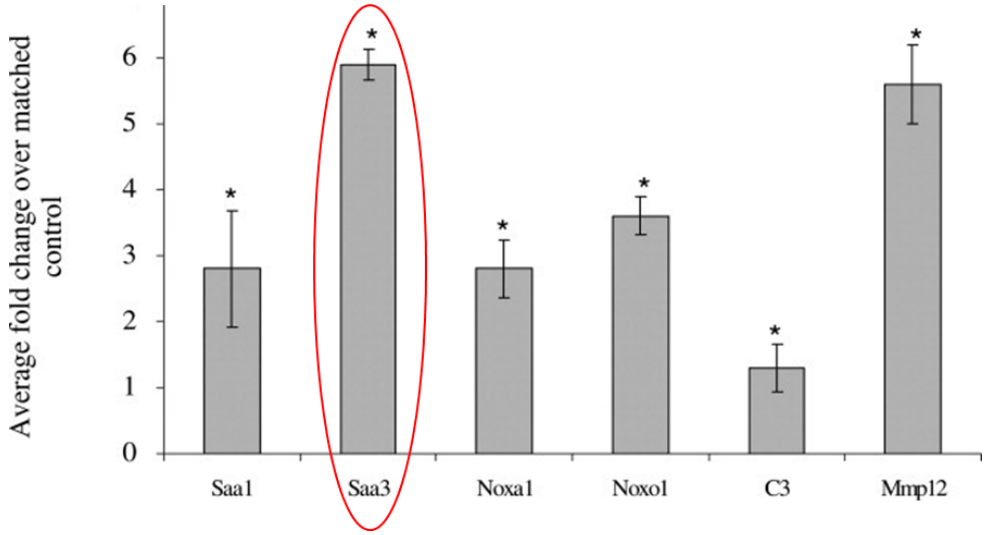


Boyadhziev et al., unpublished



Rahman et al., Mutagenesis. 2017 Jan;32(1):59-76

Finding relevant in vitro biomarkers/endpoints/assays

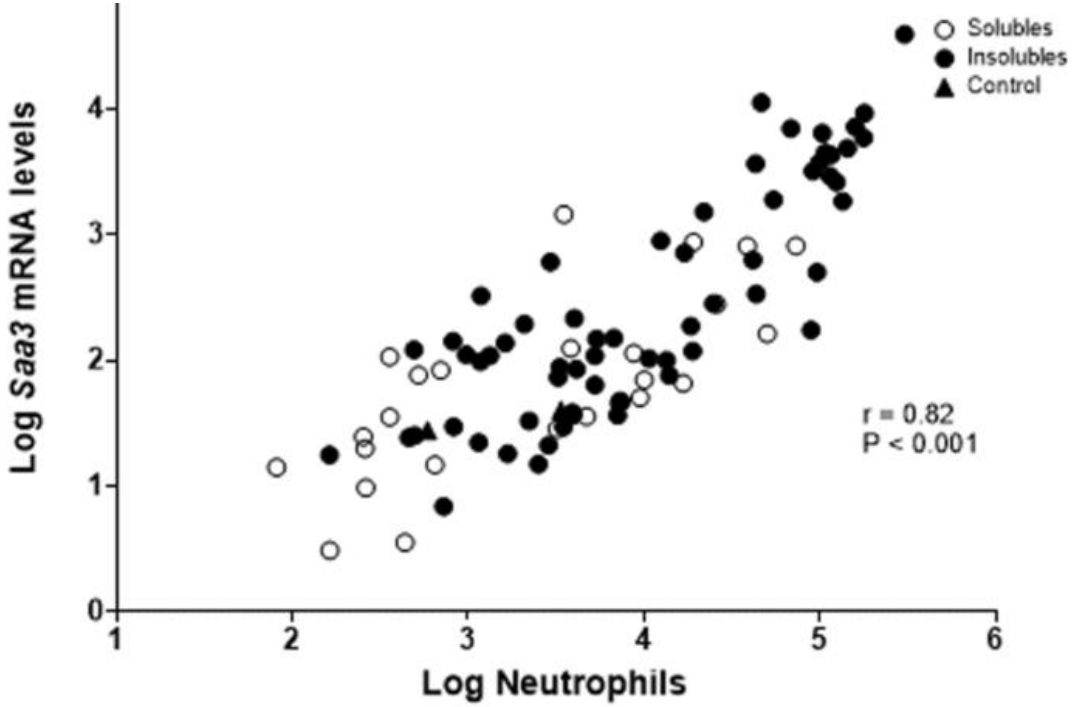


› Environ Mol Mutagen. 2011 Jul;52(6):425-39. doi: 10.1002/em.20639. Epub 2011 Jan 21.

Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: a toxicogenomic study

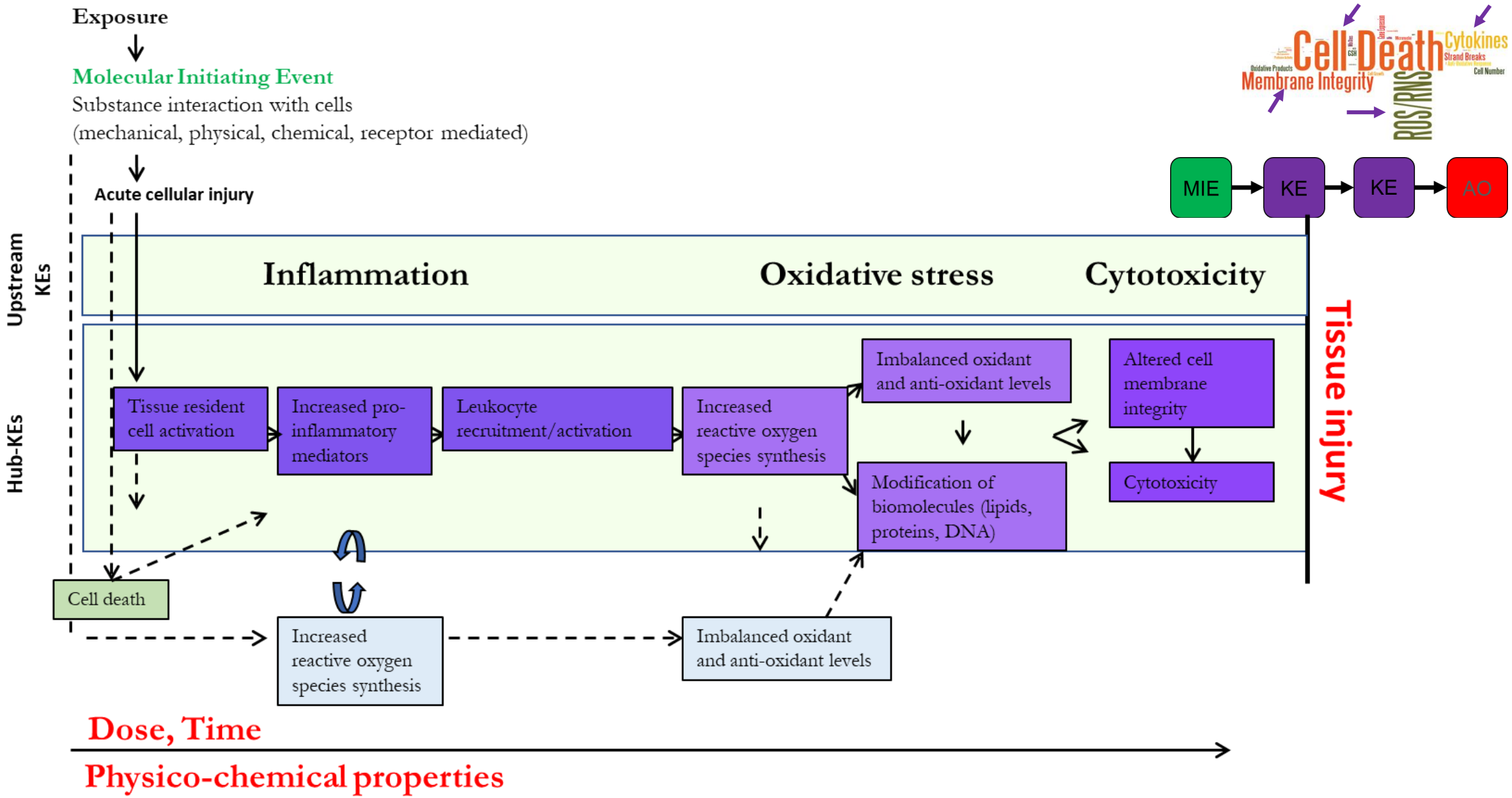
Sabina Halappanavar [1], Petra Jackson, Andrew Williams, Keld A Jensen, Karin S Hougaard, Ulla Vogel, Carole L Yauk, Håkan Wallin

Gutierrez et al., *Part Fibre Toxicol* **20**, 4 (2023). https://doi.org/10.1186/s12989-023-00514-0



What cell types can be used to assess SAA3 in vitro?

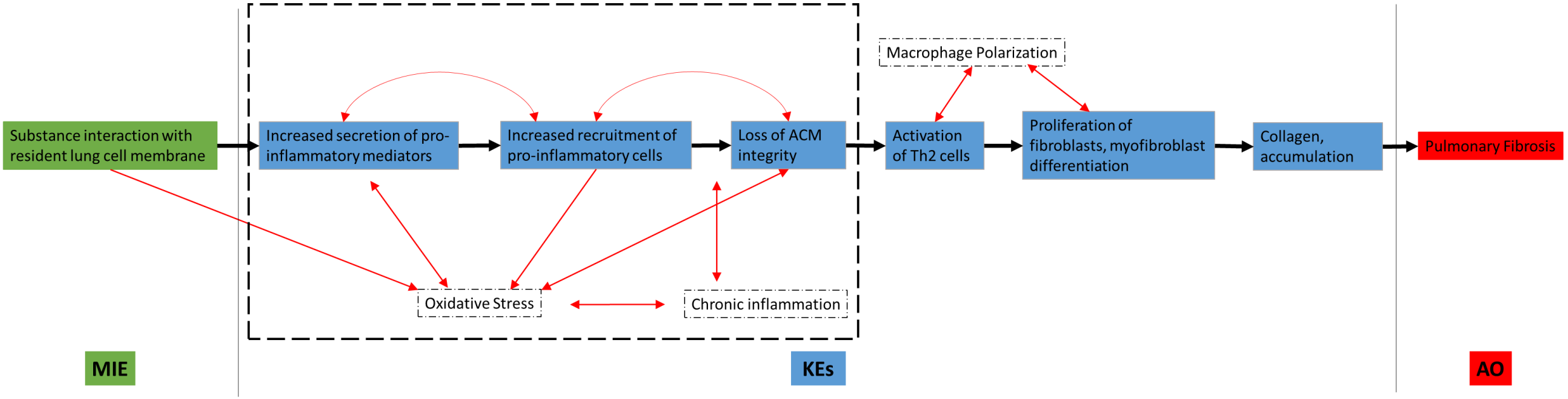
AOP framework – in vitro inflammation, oxidative stress and cytotoxicity



Halappanavar et al., Small, Volume: 17, Issue: 15, 2021

AOP 33 – previously known as AOP 173

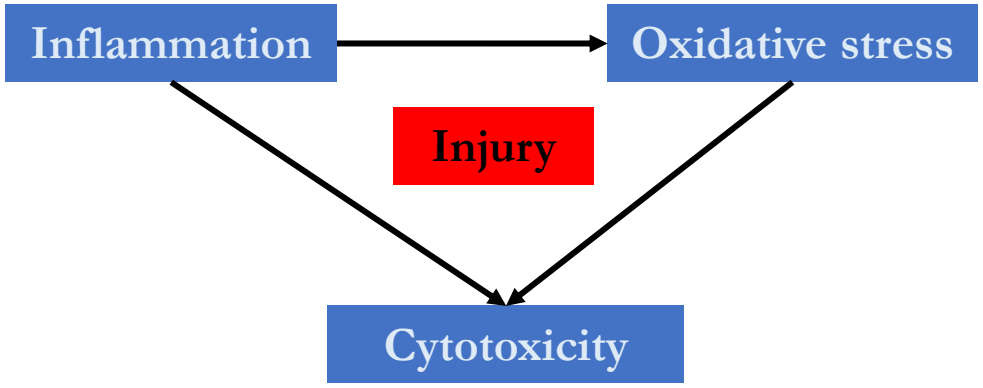
Schematic depicting how oxidative stress and macrophage polarisation connect to the main KEs in the AOP 33 through feedback loop.

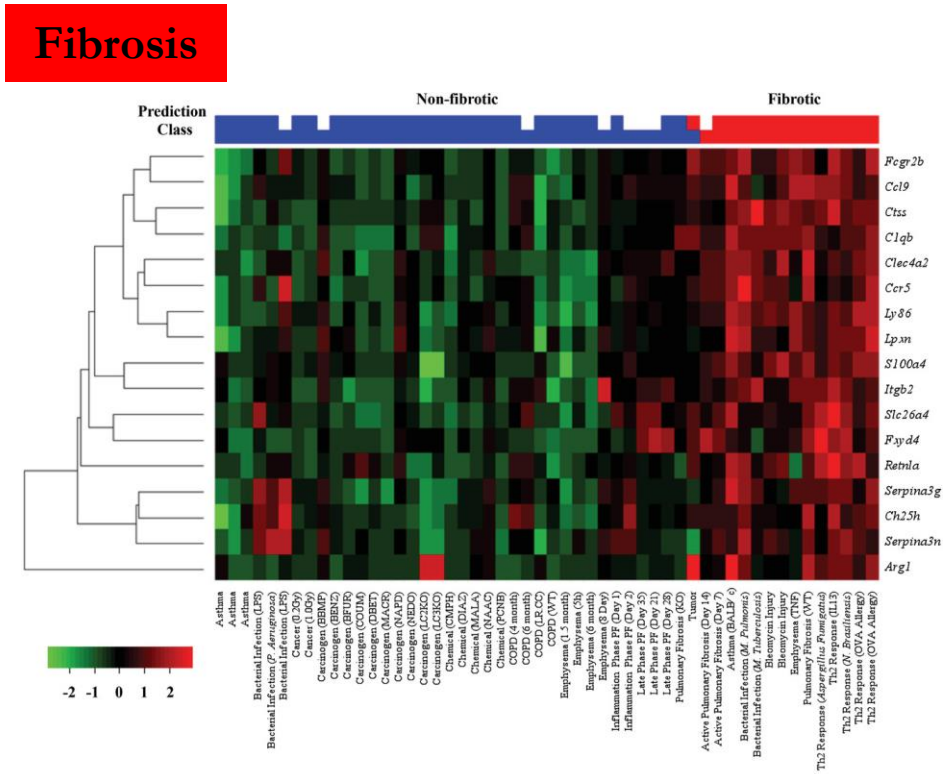


Halappanavar, S., et al. (2023), "Substance interaction with the pulmonary resident cell membrane components leading to pulmonary fibrosis", *OECD Series on Adverse Outcome Pathways*, No. 33, OECD Publishing, Paris, https://doi.org/10.1787/10372cb8-en

Finding relevant in vitro biomarkers/endpoints/assays

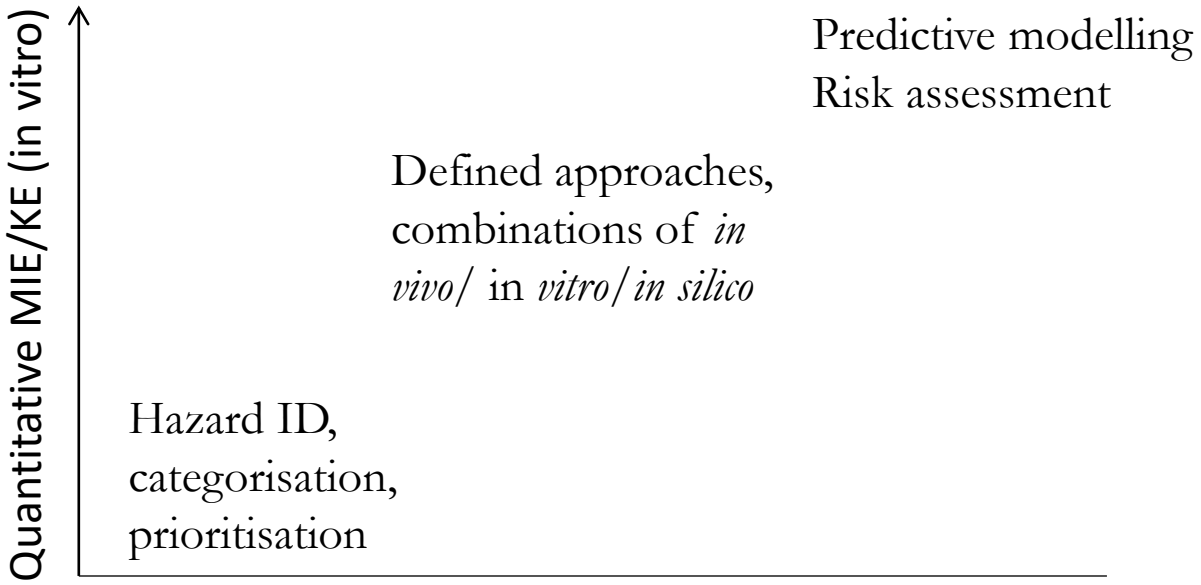
- What cell types and models?
- Tissue organoids?
- How many endpoints?
- Classification efficiency- 2/3, 3/4?





Selection of relevant cell types/biomarkers/endpoints/assays and validation Next steps

- Conduct community-wide survey (through OECD)
    - Seek assay, cell type, biomarker suggestions
    - Establish criteria for consideration
- Explore defined approaches (combination of assays with best predictive capacity)
- Test the approach - assess a set of nanomaterials
- Establish class prediction criteria



ACKNOWLEDGEMENTS

AOP 173
Monita Sharma, PETA, USA
Amy Clippinger, PETA, USA
Silvia Solorio-Rodriguez, HC, Canada
Hakan Wallin, NRCWE, Denmark
Ulla Vogel, NRCWE, Denmark

Samples, QSAR
Ulla Vogel, NRCWE, Denmark
Hakan Wallin, NRCWE, Denmark
Tomasz Putzyn, University of Gdansk, Poland
Karolina Jagiello, University of Gdansk, Poland

Genomics, Nanotoxicology and Microplastics Toxicology Laboratory, Health Canada, Ottawa, Canada

Dongmei Wu
Luna Rahman, Ph.D.
Andrew Williams – Biostatistician
Jake Nikota, Ph.D.
Sarah Labib, Ph.D.
Andrey Boyadhziev

ENV/JM/MONO(2020)35
ENV/JM/MONO(2020)34
https://www.oecd.org/

Funding

Health Canada Santé Canada

Chemicals Management Plan

Genomics Research and Development Initiative