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Adverse Outcome Pathway Guided Alternative Toxicity Testing Strategy for Lung Fibrosis

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YOUR HEALTH AND SAFETY... OUR PRIORITY.

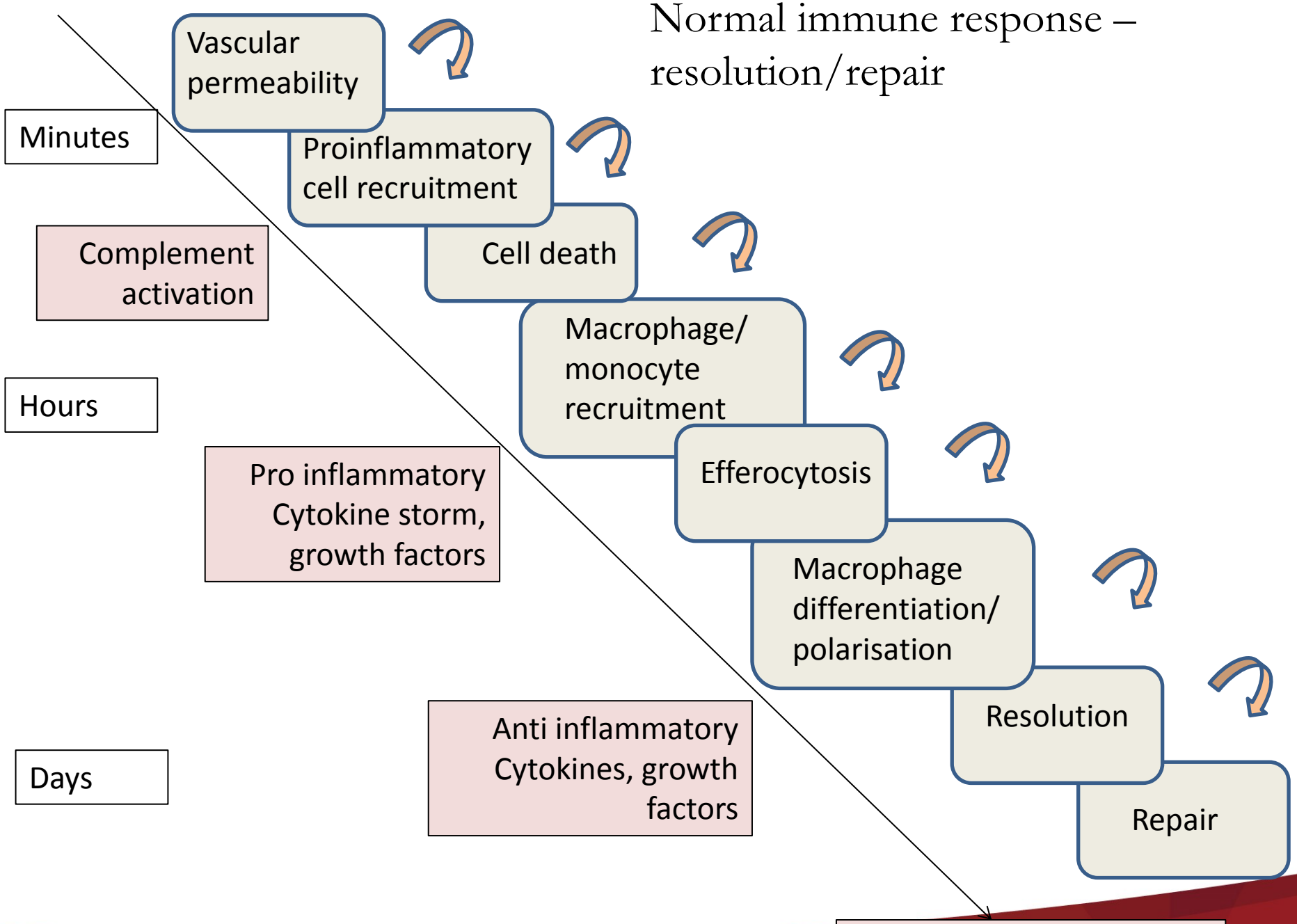
Lung fibrosis

- Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease with poor prognosis (< 5 year); etiology is unknown
- Pulmonary fibrosis is one of the adverse outcomes of exposure to environmental toxicants; occupational exposure to silica, asbestos induces pulmonary fibrosis in humans
- Understanding of the pathogenesis of IPF comes from studies conducted on toxicology of substances such as bleomycin, silica, asbestos, fluorescein isothiocyanate, or other stimuli such as radiation, known pro-fibrotic agents
- Animal models exist, the choice for studying human pulmonary fibrotic disease – collagen content, histopathology
- In vitro models – single cellular/molecular response

Fibrosis: overgrowth, hardening, and/or scarring of tissue, a result of uncontrolled deposition of extracellular matrix components such as collagen.
Final pathologic outcome of excessive collagen deposition.

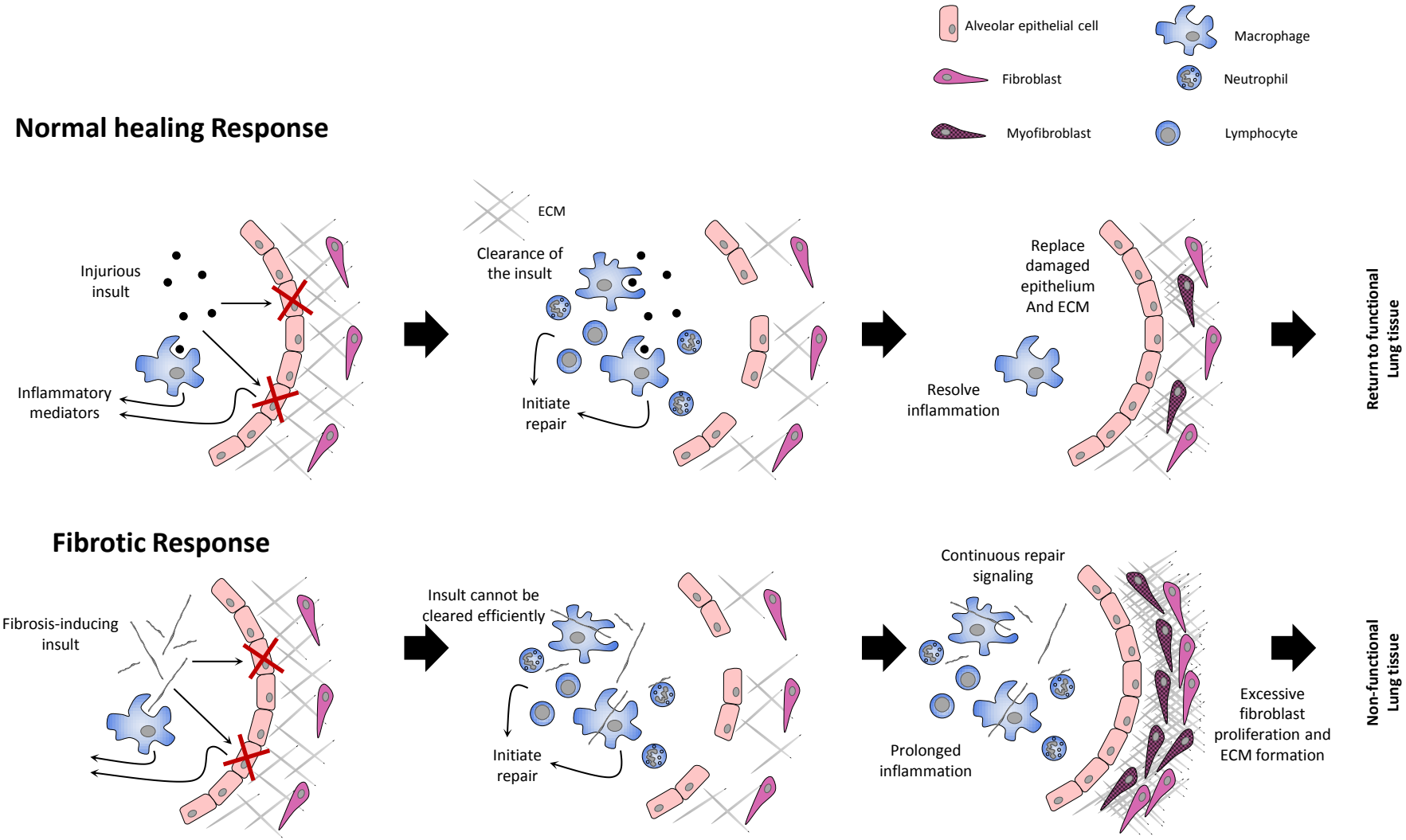
Gharaee-Kermani et al., (2005); Fibrosis Research. Methods in Molecular Medicine, vol 117. Humana Press;
Chua et al., (2005); Am J Respir Cell Mol Biol Vol 33. pp 9–13; Wynn TA, (2008); J Pathol, 214(2):199-210.

Normal immune response – resolution/repair



Reviewed in Nathan C. NATURE (2002); VOL 420: 19-26

Lung fibrotic response - mechanisms



Alternative toxicity testing methods – roadmap to hazard/risk assessment

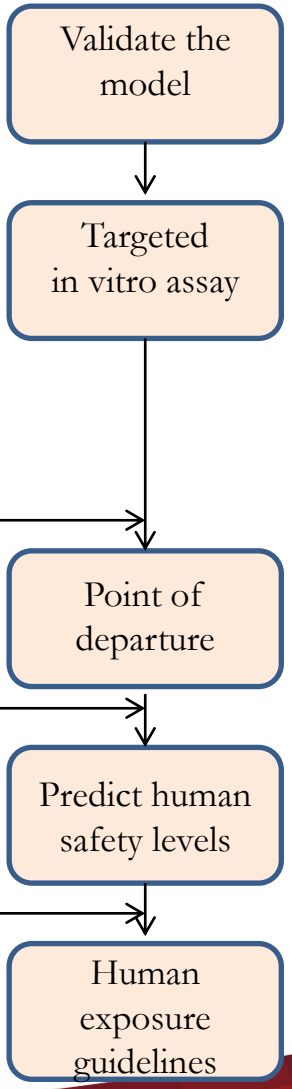
- Identify an adverse outcome – homeostasis vs adaptation vs adversity
- Establish an adverse outcome pathway – identify toxicity pathways, key events
- Characterise species differences
- What is measured and what does it reflect?
- Know its strengths and limitations
- A tested protocol
- Reproducibility (intra- and inter-)
- Performance - list of reference substances
- Performance – against an in vivo endpoint
- TG development and adoption
- Regulatory acceptance

- Pathway/apical endpoint dose-response analysis

- Systems biology, computational modelling

- Quantitative in vitro - in vivo extrapolation, reverse dosimetry

Establishing an evidence-based adverse outcome pathway is the key

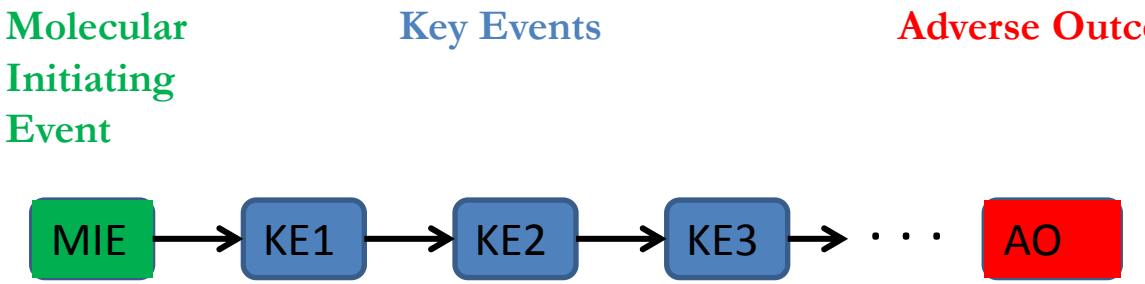


Modified from - Clewell R.A. et al., 2016. Validation of alternative methods for toxicity testing, Advances in experimental medicine and Biology 856

Adverse outcome pathways (AOPs)

- 'Conceptual constructs that portray existing knowledge concerning the linkages between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment'

  (Ankley et al 2010, Environ. Toxicol. Chem., 29(3): 730-741).



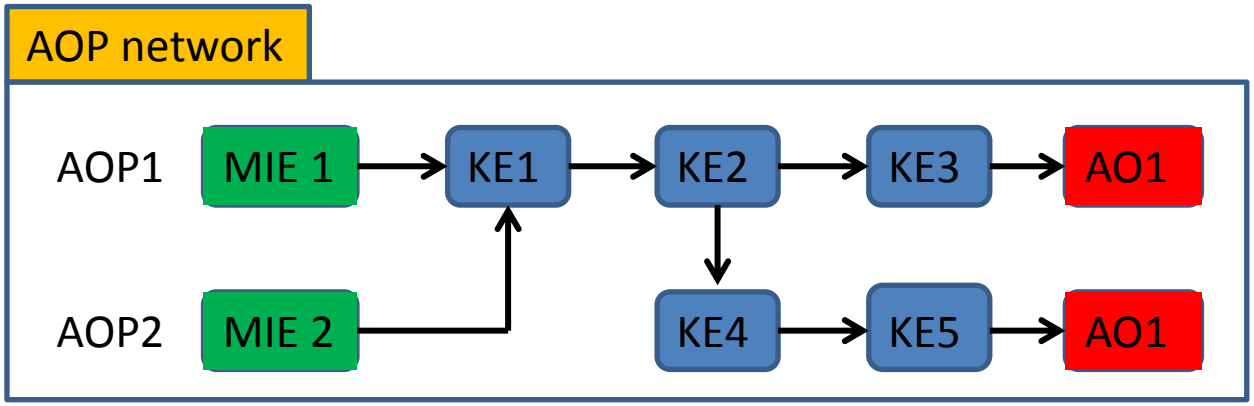
- Systematic organization - simplified representation
- Measurable or observable biological/chemical changes that are essential for toxicity
- Quantitative Key Event Relationships (KER) – biological plausibility and empirical evidence → supported by

  (Villeneuve et al 2014, Toxicological Sciences, 142(2), 312–320)

https://aopwiki.org/aops AOP Knowledge Base OECD's Series on Adverse Outcome Pathways OECD AOP users' handbook

Core principles of AOPs

- AOPs are not chemical specific
- AOPs are modular, components of AOPs (KEs and KERs) can be reused
- An individual pathway composed of a single linear sequence of KEs and KERs
- One can develop multiple AOPs that share common KEs and KERs – networks of AOPs or add branches. These will be used to represent the complexity of the toxicity – have better predictive value
- AOPs are living documents



(Villeneuve et al 2014, Toxicological Sciences, 142(2), 312–320)

In vivo lung fibrotic response – critical events

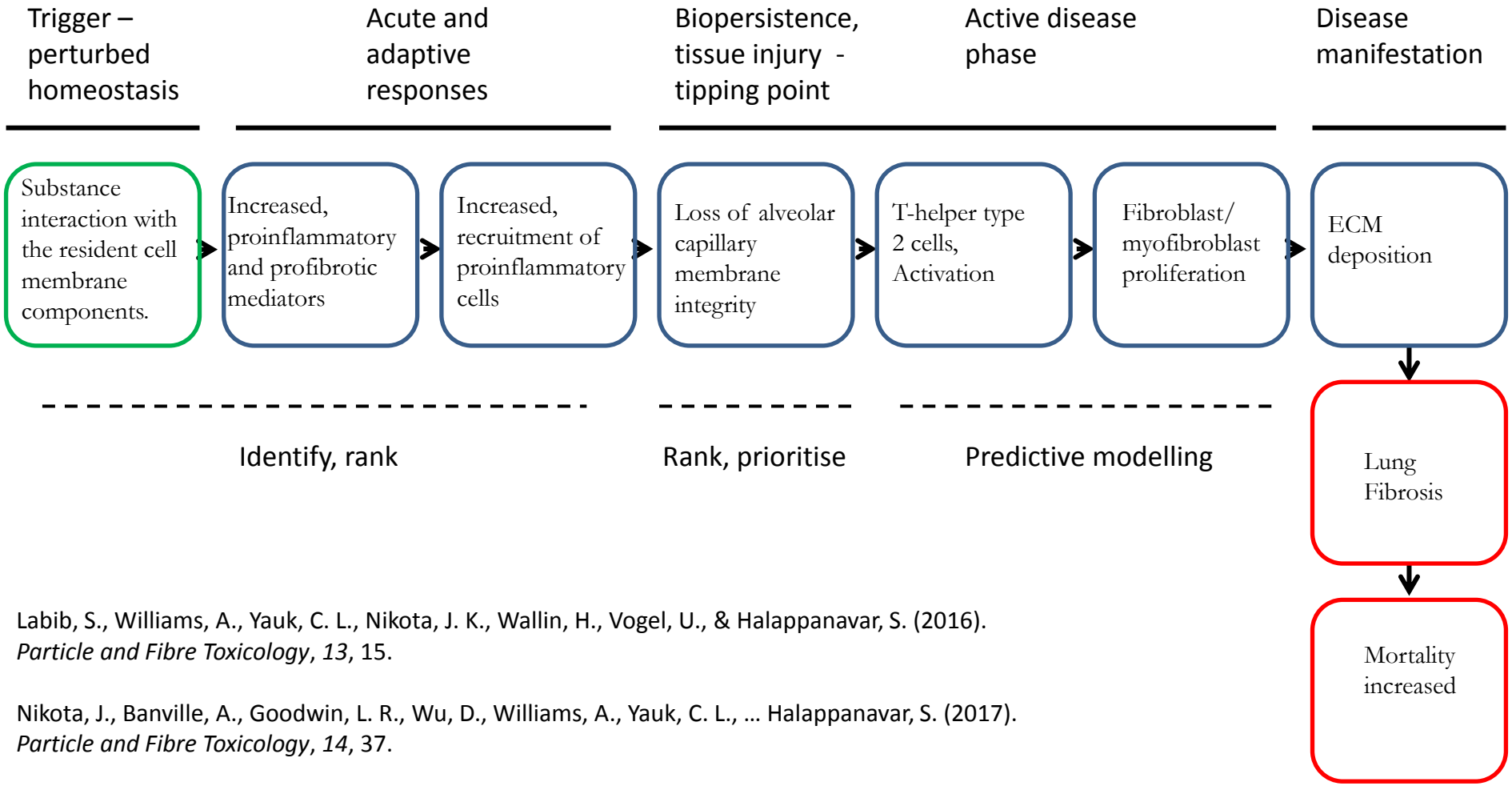
- Chronic tissue inflammation
- Persistent tissue injury
- Activation of adaptive immune response – role of Th2-type response
- The loss of alveolar-capillary barrier basement membrane
- Inability to restore the basement membrane integrity (reepithelialization/reendothelialization)
- Activation and proliferation of fibroblasts/myofibroblasts; Epithelial to mesenchymal transition
- Excessive deposition of extracellular matrix

Temporal response, Multiple cell types, Complex signalling and crosstalk , dynamic microenvironment

Strieter and Mehrad. Chest (2009); 136:1364-1370

AOP 173: Increased substance interaction with the resident cell membrane components leading to lung fibrosis - OECD EAGMS review

Sabina Halappanavar, Monita Sharma, Hakan Wallin, Ulla Vogel, Kristie Sullivan, Amy J. Clippinger
Halappanavar et al., manuscript in preparation



Labib, S., Williams, A., Yauk, C. L., Nikota, J. K., Wallin, H., Vogel, U., & Halappanavar, S. (2016). *Particle and Fibre Toxicology*, *13*, 15.

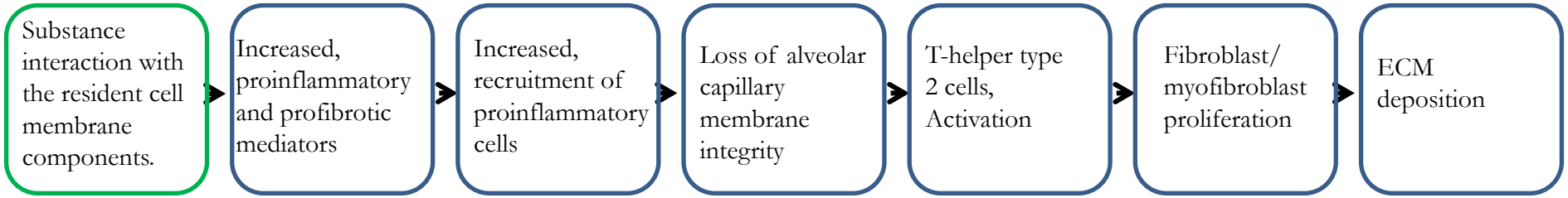
Nikota, J., Banville, A., Goodwin, L. R., Wu, D., Williams, A., Yauk, C. L., … Halappanavar, S. (2017). *Particle and Fibre Toxicology*, *14*, 37.

Williams A, Halappanavar S. (2015). *Beilstein Journal of Nanotechnology, 6:2438-2448*.

AOP 173: Increased substance interaction with the resident cell membrane components leading to lung fibrosis - OECD EAGMST internal review

In vitro assay identification

In vitro strategy considerations



Protein corona
Lysosomal uptake
DAMP release
Receptor signaling
Phys-chem

Pro inflammatory Cytokines
Pro fibrotic factors (Arg-1, TGFb1, OPN)

- Cell viability/cytotoxicity assays– not specific
- Colony forming/proliferation assay
- Loss of gap junctions
- Transepithelial electrical resistance– multi-cell type cultures
- Reactive Oxygen Species
- Lysosomal acidification
- Imbalanced proteases/antiprotease
- TNFa, IL-1b, IFNg cytokines

Th2 responsive genes/proteins

Fibroblast proliferation assays,
α-SMA
Sircol collagen assay
Hydroxyproline assay
Collagen genes I, III/proteins

Cell types? Combination of assays? Relevant combinations?

In silico? IATA

Halappanavar et al., in preparation

Conclusions

Drive to develop animal alternatives to inhalation toxicity testing

Failed efforts - replace existing animal-reliant methods by one-to-one swapping approach

Adverse outcome pathways

AOP 173 example – 'Increased substance interaction with the resident cell membrane components leading to lung fibrosis'

Mechanisms based in vitro systems - physical-chemical characteristics of substances, the complex microenvironment, cell types involved

Combination of assays, predictive modelling

Validation, adapatation