HARMLESS

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Engineered nanomaterial-relevant AOPs; network creation and identification of key nodes for adverse outcomes

Sarah Søs Poulsen, NRCWE

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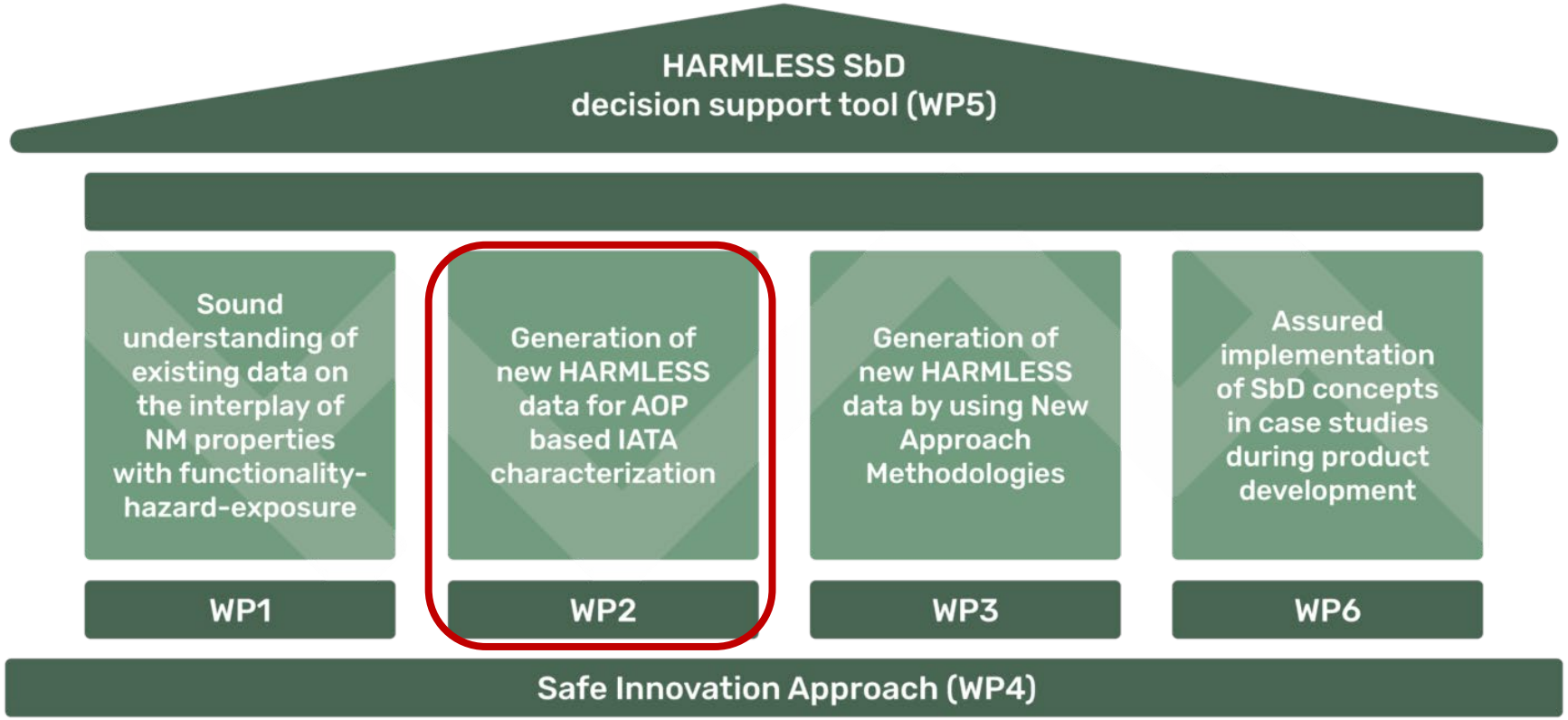
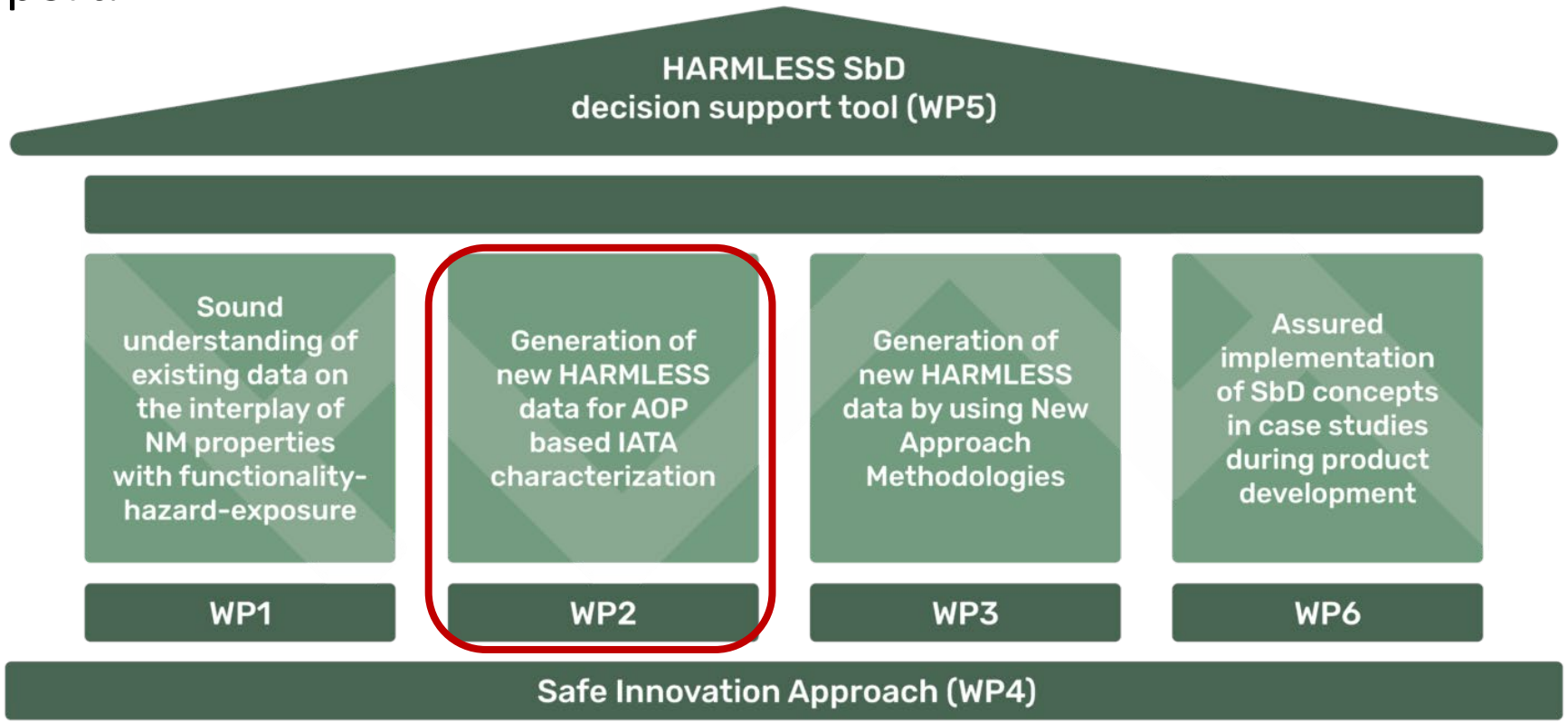
Background - HARMLESS

HARMLESS is an EU-funded H2020 Research & Innovation Action addressing Safe-by-Design of multicomponent nanomaterials
- running from January 2021 - January 2025



Background - HARMLESS

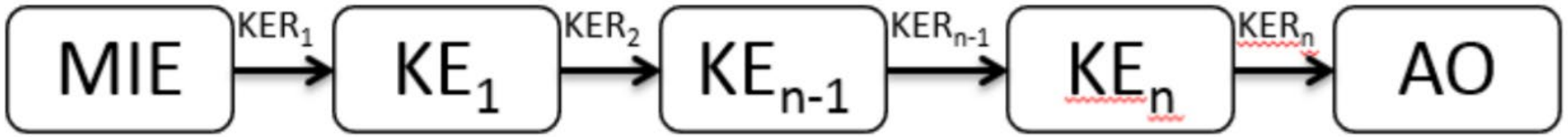
As a task, we compiled and organized existing and tentative AOPs relevant for engineeered nanomaterial-induced adverse outcomes to human health and ecology.
- Use in phys-chem correlation analyses and NAM development.
- Public report.



Background - AOPs

Adverse Outcome Pathways (AOPs) are a useful framework for organizing and presenting scientific data related to toxicological processes.

AOPs are structured representation of biological events leading to adverse effects and is considered relevant to risk assessment.



The AOP links in a linear way existing knowledge along one or more series of causally connected key events (KE) between two points — a molecular initiating event (MIE) and an adverse outcome (AO) that occur at a level of biological organization relevant to risk assessment. The linkage between the events is described by key event relationships (KER) that describe the causal relationships between the key events.

Methods
- Identification

Nano-relevant AOPs were identified and compiled from:

- other EU projects (e.g. SmartNanoTox and PATROLs).
- through identification in the AOPwiki.
- other approaches.
  - through articles.

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Methods
- Identification in the AOPwiki

Two manual searches.

AOPs with ENM-relevant AOs:

Lung inflammation
Lung fibrosis
Lung cancer
Mesothelioma
Lung emphysema
Acute inhalation toxicity
Liver inflammation
Liver fibrosis
Liver cancer
Plaque progression

ENM-relevant stressors and their AOPs:

No.	Stressor name
224	Nanoparticles
232	Iron compounds
252	Silver nanoparticles
253	UV-activated Titanium dioxide nanoparticles
254	Silica nanoparticles
255	Graphene oxide nanoparticles
305	Single walled carbon nanotubes
306	Multi-walled carbon nanotubes
318	Carbon nanotubes
338	Carbon nanotubes, Multi-walled carbon nanotubes, single-walled carbon nanotubes, carbon nanofibres
339	Carbon nanotubes, Multi-walled carbon nanotubes, Single-walled carbon nanotubes, carbon fibres
357	Titanium oxide (TiO)
375	Stressor:338 Carbon nanotubes, Multi-walled carbon nanotubes, single-walled carbon nanotubes, carbon nanofibres
377	Insoluble nano-sized particles
528	PM 2.5
622	Cationic polyamidoamine dendrimer (nanoparticle)
634	Nanomaterials
660	PM10

Methods
- Selection

This resulted in a list of **25 ENM-relevant AOPs for human toxicology** and **7 ENM-relevant AOPs for ecotoxicology**.

Selection criteria were discussed by the HARMLESS consortium.

For human toxicology, AOPs not focused on pulmonary exposure and oral exposure, and not targeting the lung or liver were excluded from the AOP list, resulting in a final list of 20 AOPs.

No selection based on AOP status was conducted.

LUNG	**No. AOPs**
Pulmonary/Lung fibrosis (AO1458/AO1276)	5
Lung cancer (AO1670)	2
Decrease, Lung function (AO1250)	3
Increased, mesotheliomas (AO1090)	2
Hyperinflammation (AO1868)	1
Lung emphysema	1
LIVER	**No. AOPs**
Liver fibrosis (AO344)	2
Formation, Hepatocellular and Bile duct tumors (AO856)	1
Increase, hepatocellular adenomas and carcinomas (AO719)	1
Hepatotoxicity (AO1291)	1
OTHER	**No. AOPs**
~~Sensitisation, skin (AO827)~~	~~1~~
Plaque progression in arteries (AO1443)	1
~~Reproductive failure (AO1277)~~	~~3~~
~~Neurodegeneration (AO1514)~~	~~1~~

Methods
- Network creation

Networks were created based on AOs:

- Lung fibrosis
- Lung cancer/mesothelioma
- Decreased lung function
- Changes in the liver

To visualizing relationships and nodes.

To identify important KEs and subsequently, potential predictive *in vitro* biomarkers/assays.

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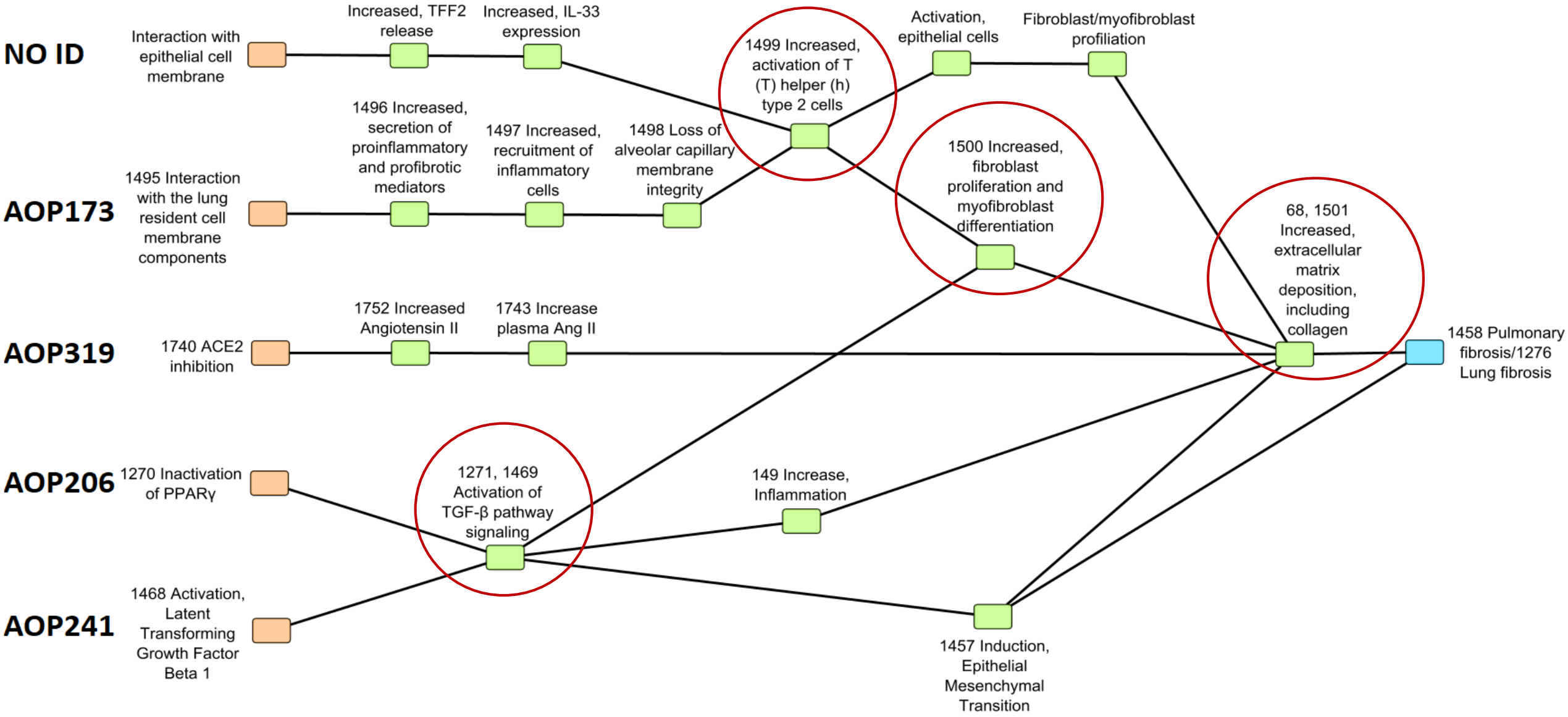
Results
- Lung fibrosis network



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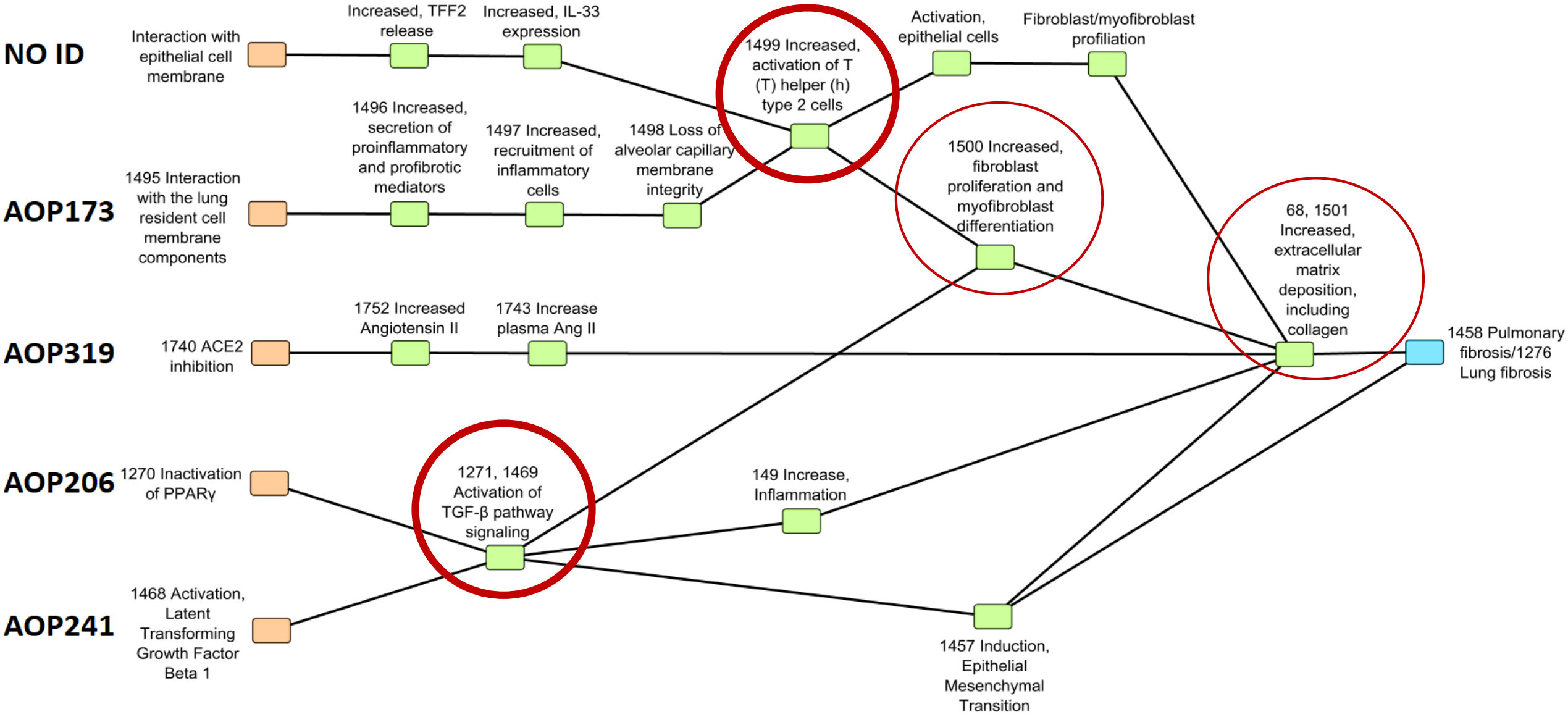

Results
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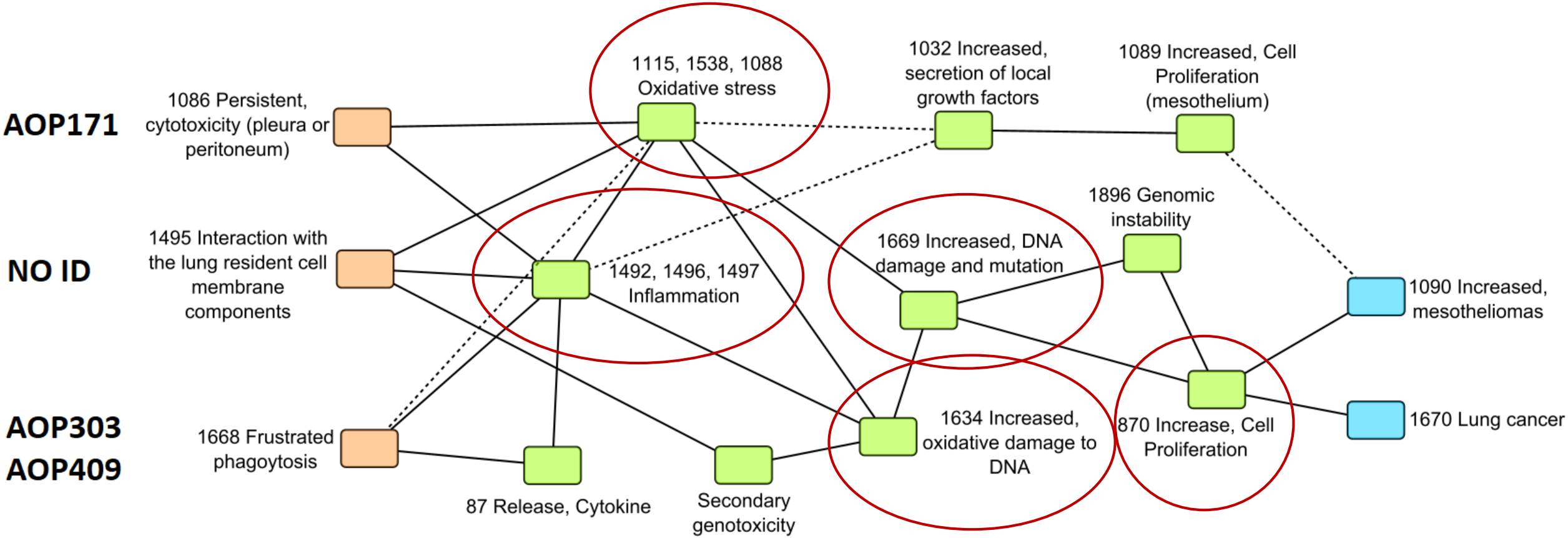
Two early nodes:
Activation of TGF-β pathway signalling (KE1271/KE1469) and Increased, activation of T (T) helper (h) type 2 cells (KE1499)

- **TGF-β** pathway signalling **stimulates** activation and proliferation of **fibroblasts** and **myofibroblasts** during tissue repair.
  TGF-β inhibits on the expression of **matrix metalloproteases** and stimulate their inhibitors. Both processes **enhance** the **fibrotic process** by accumulation of ECM materials.

- **Th2 cells** are differentiated from **naïve CD4+ T cells**, primarily under the influence of **IL-4** produced by antigen presenting cells.
  Th2 cytokines are mainly produced by Th2 cells themselves.
  Mast cells, macrophages, epithelial cells and activated fibroblasts also produce Th2 cytokines upon appropriate stimulation.
  It has been shown that **expression of Th2 cytokines** is **crucial** for **fibrosis development** in mice, despite a highly active Th1 response.

Results
- Lung cancer/mesotheliomas network



Overall, the network was more intertwined than the fibrosis network.
Nodes were more generalized than for fibrosis.

Conclusions and perspectives

- Identification and overview of ENM-relevant AOPs for human toxicology and ecotoxicology.
- Combined AOPs and created networks for 4 major adverse outcomes for human toxicology.
- Identification of network nodes representing central, upstream KEs for progression towards the AOs.

  - The main KE nodes identified were related to inflammation (lung fibrosis, lung cancer/mesothelioma, and liver fibrosis networks), oxidative stress and oxidative DNA damage (cancer/mesotheliomas network), and ECM and collagen deposition (lung and liver fibrosis networks).

- Potential predictive *in vitro* biomarkers/assays.
- Report publically available.

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Mario Pink

To you!

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