Multi-omics approach for identification of key events (KEs) in NM-related AOP development

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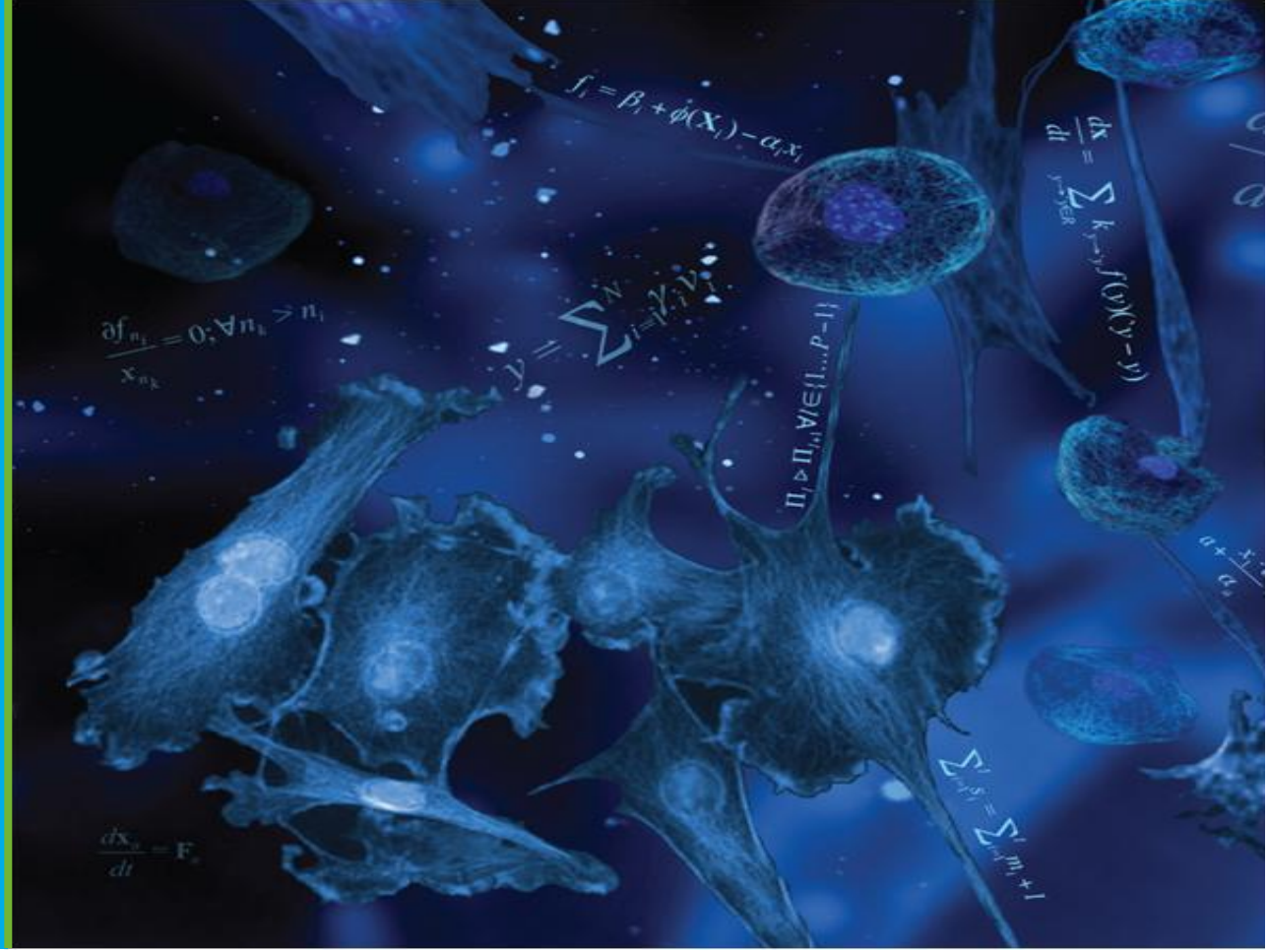
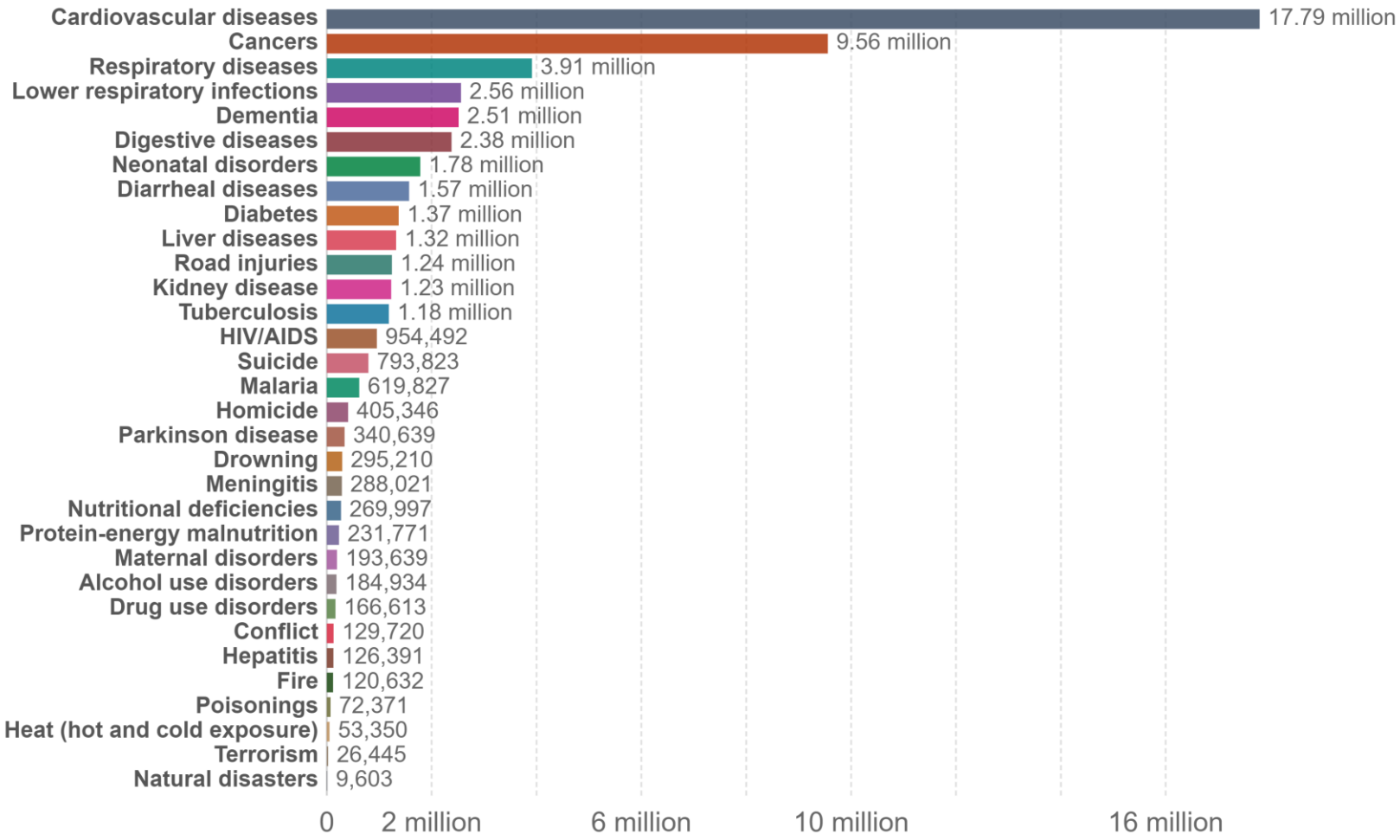


SmartNanoTox
Smart Tools for Gauging Nano Hazards

SYSTEMS BIOLOGY IRELAND

UCD DUBLIN

Number of deaths by cause, World, 2017



Source: IHME, Global Burden of Disease

OurWorldInData.org/causes-of-death • CC BY

Outline

- Introduction
- Methods
- AOPs
  - Inflammation/Cardiovascular disease
  - Cancer
  - Fibrosis

WP3

- Task 3.2. Perform transcriptomics (and targeted proteomics) analyses of in vivo and in vitro exposures of mice and rats to the NMs. Identification of the MIE/KE for each toxicity pathway or adverse outcome pathway.

- Task 3.3 Integration and the analyses of experimental data by statistical models and prediction of toxicity pathway using omics data.

Our data analysis pipeline

Row data Preprocessing → Analysis and biological interpretation → Reports Publications

Preprocessing

Row data

Microarrays
RNA-seq
single cell RNA-seq
Mass spectrometry data

Differentially expressed genes/proteins

limma
DESeq2
SEURAT

Pathways analysis

ORA

SPIA

KEGG
REACTOME
MSigDB
GO

GSEA

Motif analysis

Network analysis

PPI
co-expression

PCA

MOFA

Supervised and unsupervised machine learning

Random Forest

Project reports

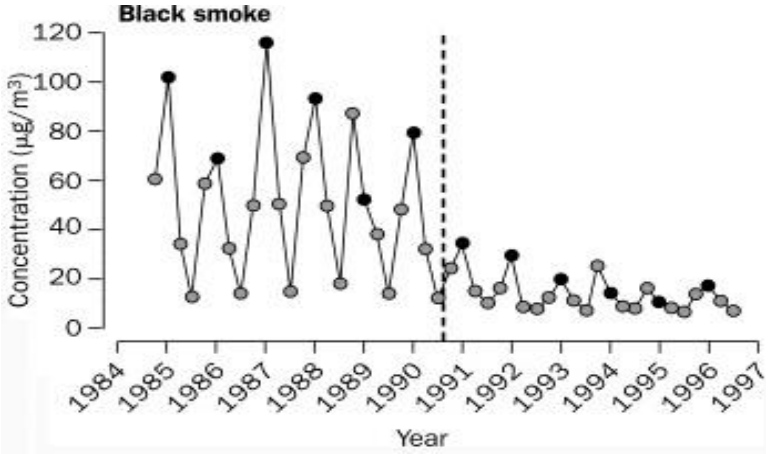
Interactive web applications

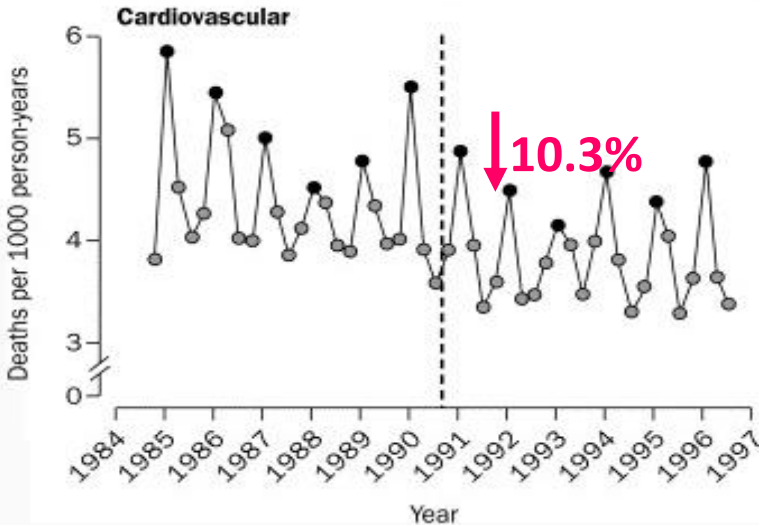
Inflammation
Cardiovascular diseases

THE LANCET

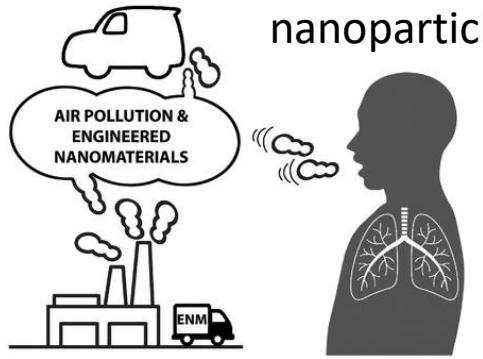
Effect of air-pollution control on death rates in Dublin, Ireland: an intervention study. The Lancet, October 2002.

On Sept 1, 1990, the Irish Government banned the marketing, sale, and distribution of black coals within the city of Dublin

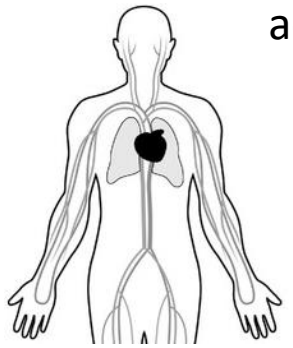




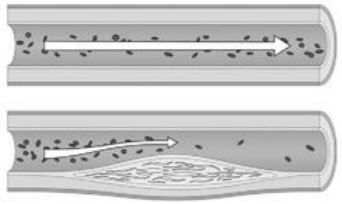
Inhalation of nanoparticles



Systemic circulation acute phase proteins



Atherosclerosis plague progression



Niels Hadrup, , Vadim Zhernovkov, et al. Small, March 2020

Top ranked acute phase response genes and KEs predicted by random forest regression model

Ulla Vogel, NRCWE, Denmark

Tobias Stoeger, HMGU, Germany

- In total, 26 different types of NMs and more than 1200 samples from transcriptomics datasets were analysed. Neutrophil infiltration values were used as marker of acute phase response and chronic inflammation .
- We analyzed global gene expression changes occurred at the early stage of NM treatment and used level of neutrophil infiltration for:
  - days 1-7 (Acute phase response AOP)
  - day 28 (Chronic inflammation AOP)

	Label	***Features***			
Group of samples	Neutrophiles (%)	Gene 1	Gene 2	Gene 3	...
GSE46998 Mitsui7 Dose 162µg day 3	80	1	0	3.5	
GSE35193 CB Dose 162µg day 1	10	1.5	2	0.9	
GSE55286 NM401 Dose 162µg day 28	70	0.6	0.2	0	
...					

scikit-learn python library

- Identified biomarkers, MIE and KEs for the acute phase response AOP:
  - Release of SAAs, TLRs signaling. Saa1, Saa2, Saa3, Nfkbie, Cd14
  - Iron metabolism. Lcn2, Fth1, Hpx
  - Mitochondrial DAMPs. Interferon response.
  - Disruption of lysosome integrity. Marker: Cstb. The protein plays a protective role against lysosomal proteases that are released into the cytosol after nanoparticle-induced lysosomal disruption.
  - Hypoxia and oxidative stress. Rbm3, Glrx
- Chronic inflammation AOP
  - Th2 response. Markers: Nfil3, Adm, Areg, Bcl3, Ccl17

Niels Hadrup, Vadim Zhernovkov, et al. Small, March 2020

Cancer

Key characteristics of carcinogens

Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

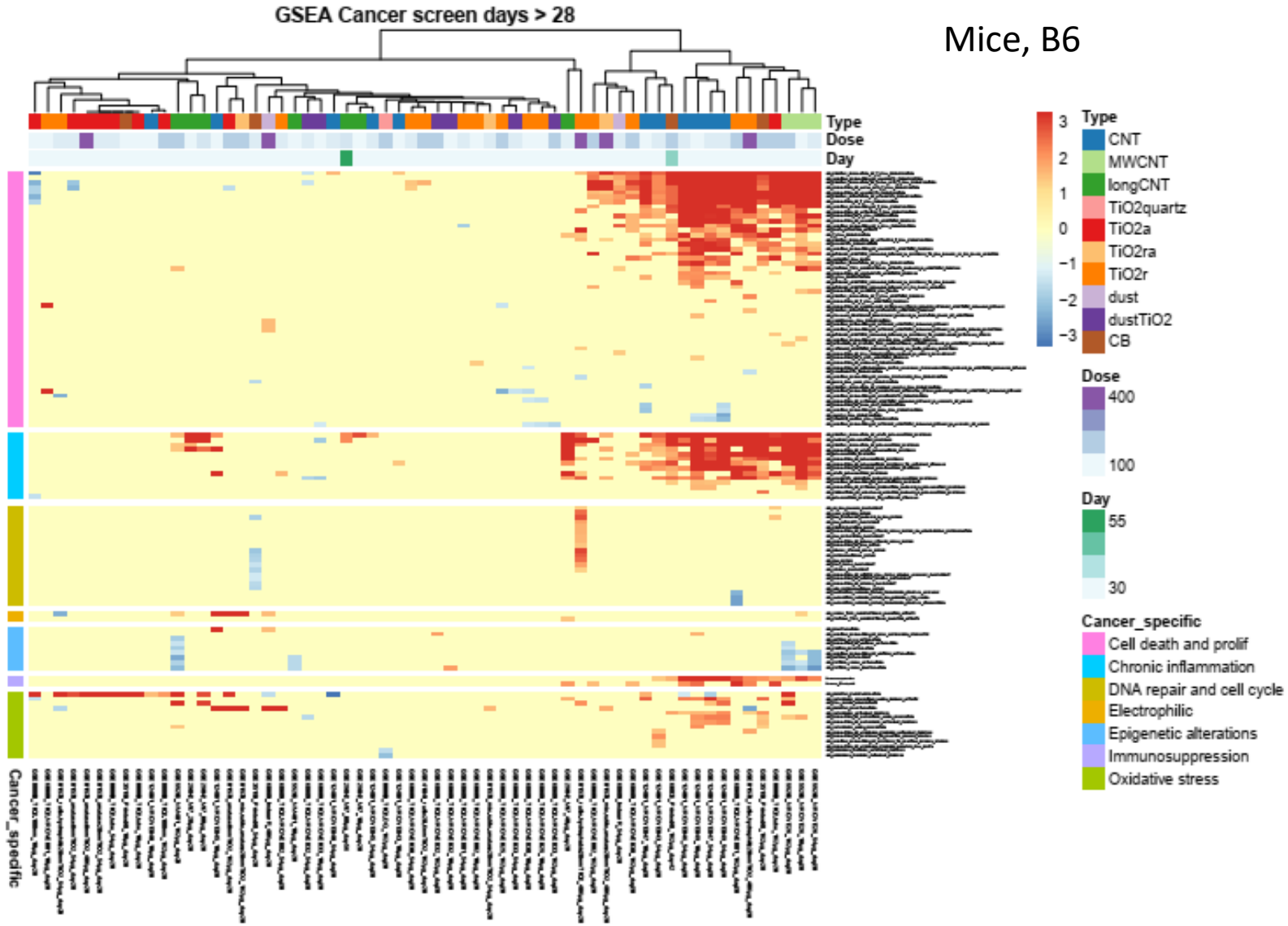
Human carcinogens commonly show ≥ 1 of the 10 key characteristic

Martyn T. Smith, et al. Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis.

Prediction of cancer risk based on key characteristics of carcinogens

Ulla Vogel, NRCWE, Denmark

- Alters cell proliferation or cell death
- Induces chronic inflammation
- Alters DNA repair
- Act as an electrophile
- Induces epigenetic alterations
- Is immunosuppressive
- Induces oxidative stress



Prediction of cancer risk based on key characteristics of carcinogens

Ulla Vogel, NRCWE, Denmark

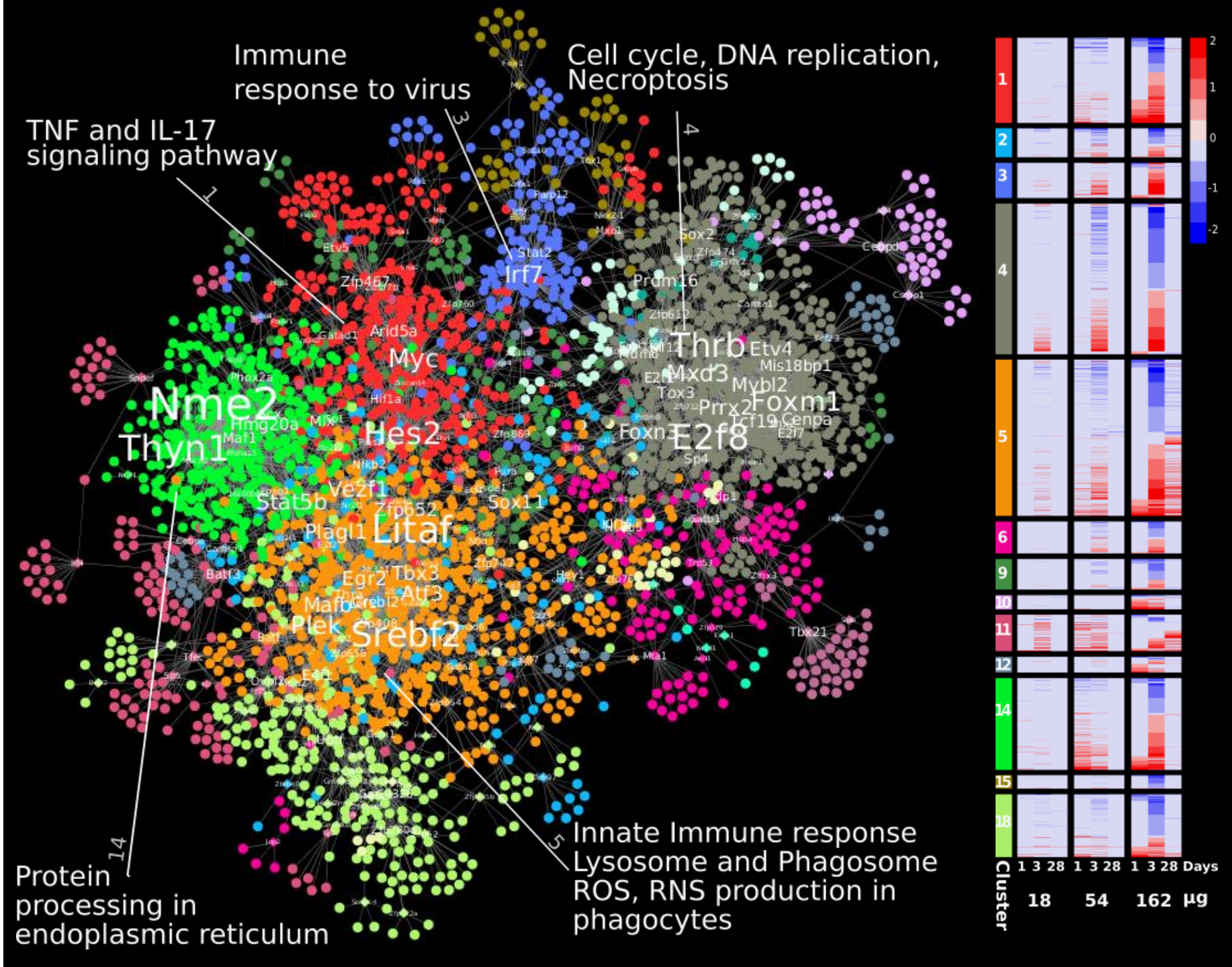
key characteristics	TIO2 tube 162µg	Printex90 162µg	NRCWE26 162µg	hydrophilic 20nmTIO2 486µg	TIO2 NRCWE001 162µg	CNT NRCWE049 54µg
Cell death and proliferation	+++	++	+++	+++	+++	+++
Chronic inflammation	+++	+++	+++	+++	+++	+++
DNA repair and cell cycle						
Electrophilic						
Epigenetic alterations			+			
Immunosuppression	+++	+	+	++	+++	++
Oxidative stress		++	+++	+		++

Fibrosis

Network analysis

- Transcriptomics data: GSE55286 Agilent microarrays [Poulsen et al. 2015]
- Mice. Doses: 18, 54, 162 µg. Post exposure time: 1, 3, 28 days
- Nanoparticles: NRCWE-26 (847±102 nm), NM-401 (4048±366 nm)

Zhernovkov, et al, 2019



An Integrative Computational Approach for a Prioritization of Key Transcription Regulators Associated With Nanomaterial-Induced Toxicity

Vadim Zhernovkov,* Tapesh Santra,* Hilary Cassidy,* Oleksii Rukhlenko,* David Matallanas,*,† Aleksandar Krstic,* Walter Kolch,*,†,‡ Vladimir Lobaskin,§ and Boris N. Kholodenko*,†,‡,¶,1

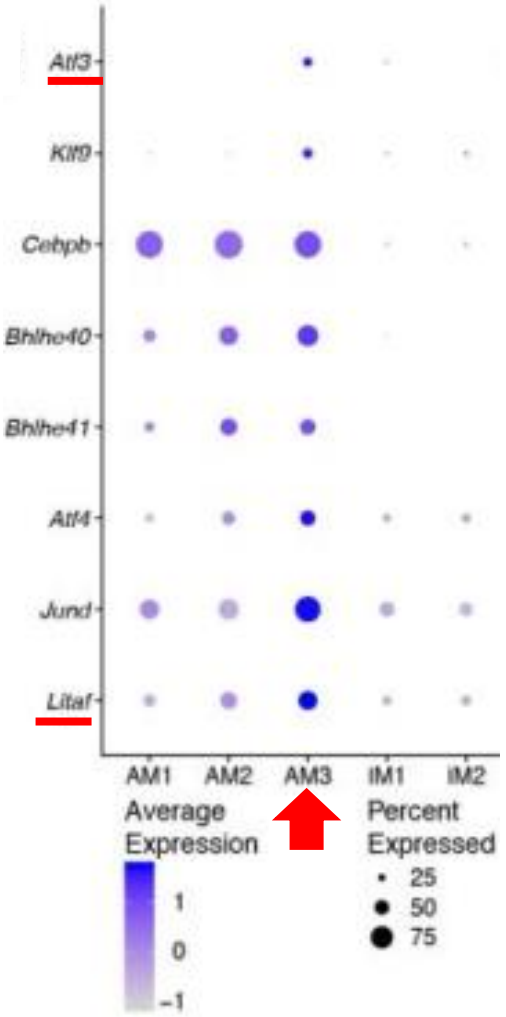
*Systems Biology Ireland; †School of Medicine and Medical Science; ‡Conway Institute of Biomolecular & Biomedical Research; §School of Physics, University College Dublin, Dublin 4, Ireland; and ¶Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06520

List of predicted regulators: Litaf, Atf3, Mafb, Batf3/Batf, Thyn1, Hif1a, Egr2, Srebf2, Nme2-Myc-Mxd1

EUROPEAN RESPIRATORY *journal*
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Joshi N, et al. A spatially restricted fibrotic niche in pulmonary fibrosis is sustained by M-CSF/M-CSFR signalling in monocyte-derived alveolar macrophages. Eur Respir J. 2020 Jan

- Methods: single-cell RNA-seq and spatial transcriptomics, asbestos-induced pulmonary fibrosis.
- Distinct populations of macrophages differentially contribute to organ fibrosis.
- Tissue-resident alveolar macrophages, tissue-resident peribronchial and perivascular interstitial macrophages and monocyte-derived alveolar macrophages are present in the fibrotic niche. Deletion of monocyte-derived alveolar macrophages but not tissue-resident alveolar macrophages ameliorated asbestos-induced lung fibrosis.



Integrated analysis of transcriptomics and proteomics experimental data

Carole Seidel,
INRS, France

NM-401
NM-403

in vivo
lung rats

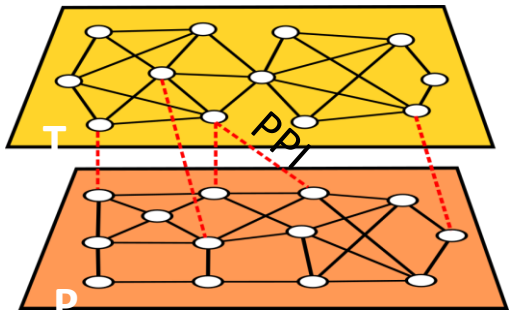
Laurent Gaté,
INRS, France

Microarrays

David Gomez,
SBI UCD

Proteomics
Data
BALF

Network-based Analysis

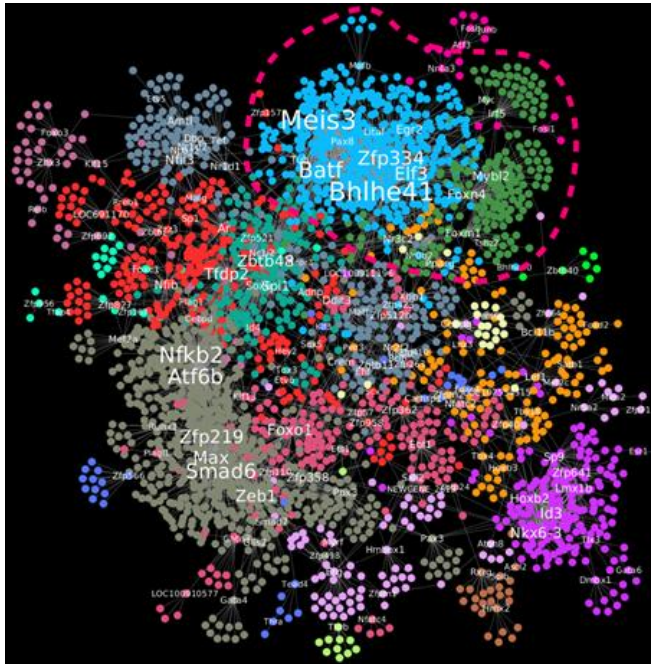


Multi-Omics Factor Analysis

	genes	proteins
Total variance explained		
R2	0.5835739	0.4392815
Variance explained per factor		
LF1	**0.25281762**	**0.07413559**
LF2	0.14407684	0.07718124
LF3	0.04300956	0.16087108
LF4	0.01450957	0.08469575
LF5	0.04164878	0.03365491
LF6	0.03526747	0.01387117

MOFA R/Bioconductor package

Regulators:
Litaf
Foxm1
Myc
Egr2
Mafb



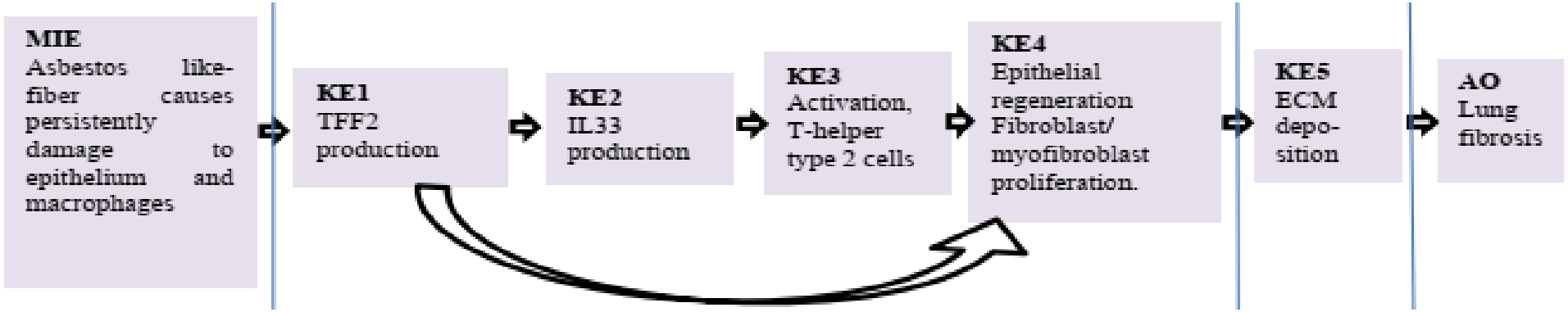
Integrative transcriptomics/proteomics analysis identified that NM-401 triggered **oxidative stress defence processes**, which might be considered as a major component of the mode of action of this CNT.

Carole Seidel, Vadim Zhernovkov, et al

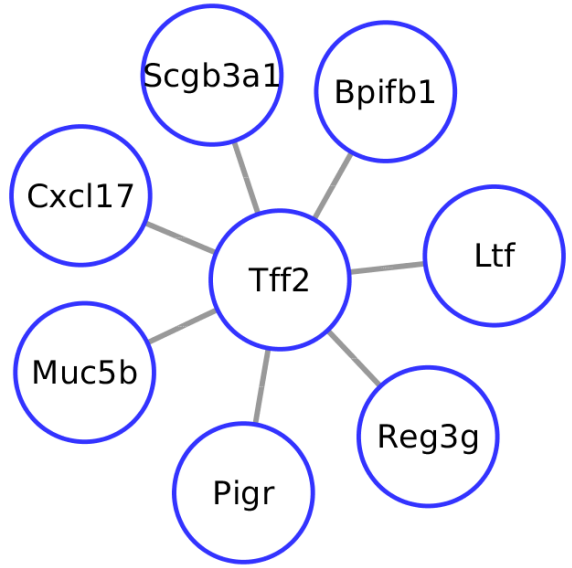
AOP: Interactions with lung tissues leading to lung fibrosis via a pathway involving Trefoil Factor 2

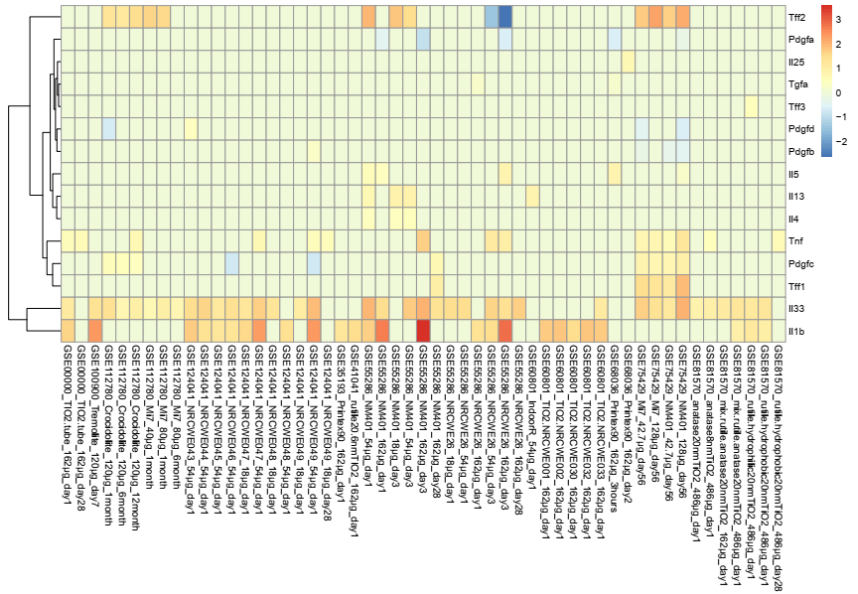
Henrik Wolf,
FIOH, Finland

Tobias Stoeger,
HMGU,
Germany



Co-expression pattern for Tff2
Algorithm: CLR
Number of samples: 3000

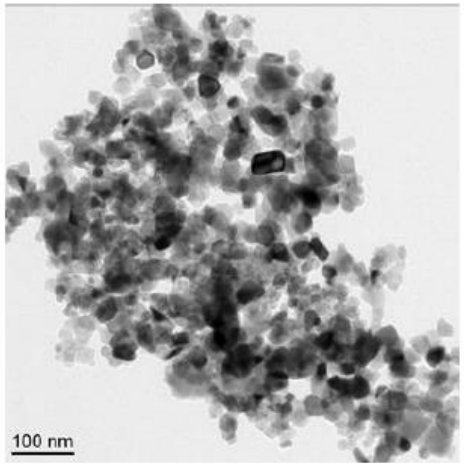


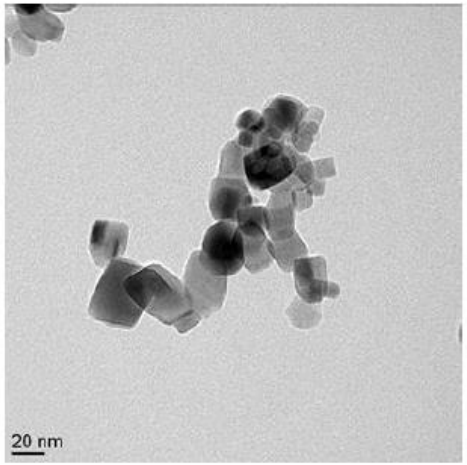


Comparison of *in vitro* and *in vivo* exposure

Olivier Joubert, University of Lorraine, France

- Air-liquid interface exposure (ALI), *in vitro* and *in vivo* exposures.
- NR8383 cells, Rats.
- TiO2 NM-105





TEM images of titanium dioxide nanoparticles (NM-105).

Gene Set Enrichment Analysis (GSEA)

Gene set	P-value in vivo Lung	P-value in vitro ALI	P-value in vitro submerged
Common gene sets between the three exposures			
IL6_JAK_STAT3_SIGNALING	1,25E-07	7,42E-02	3,82E-02
MYC_TARGETS_V2	4,30E-06	7,42E-02	3,65E-02
Common gene sets between in vivo and ALI exposures			
E2F_TARGETS	1,04E-14	8,40E-07	-
G2M_CHECKPOINT	7,96E-11	7,00E-03	-
MYC_TARGETS_V1	1,08E-04	7,00E-03	-
EPITHELIAL_MESENCHYMAL_TRANSITION	9,03E-02	3,84E-02	-
Common gene sets between in vivo and in vitro submerged exposures			
UV_RESPONSE_DN	6,21E-03	-	6,73E-02
TNFA_SIGNALING_VIA_NFKB	9,43E-03	-	1,46E-03

Mélanie M. Leroux, et al

Summary

- The network analysis and ML are powerful tools for analysis omics data and can generate biologically meaningful predictions in the nanotoxicology field.
- Open source software (R, Python).

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Otmar Schmid

Laurent Gaté
Carole Seidel

Janez Štrancar