Inhaled carbon nanotube-induced gene expression profile in rat lung

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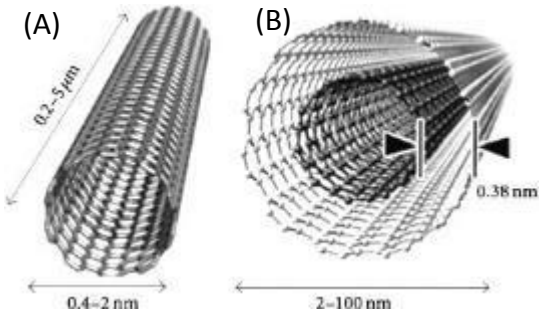
Carbon nanotubes

Unique physical properties
- **Mechanical**
- **Electrical**

Large variety of CNTs
- **Number of “walls”**
- **Length, diameter**
- **Functionalization**

Industrial and medical applications
- **Nanoelectronics**
- **Composite materials**
- **Sport goods**
- **Hydrogen storage**
- **Drug carriers**

Toxicological properties
- **Pulmonary inflammation**
- **Granuloma, pulmonary fibrosis**
- **Mesothelioma**
- **Lung cancer**



Objectives

- **Study of carbon nanotube toxicity**
  - **Sub-acute nose-only inhalation study in rats**
  - **Conventional approach**
  - **Molecular approach (High content screening method: transcriptomics)**
  - **NM-401**
  - **NM-403**

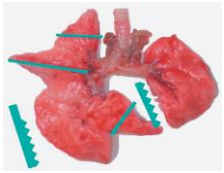
Experimental protocol

Female Sprague Dawley Rats

Nose-only inhalation exposure

(2x3h) / day,
5 days / week,
4 weeks

Control
+ 2 concentrations (0.5 and 1.5 mg/m^3)



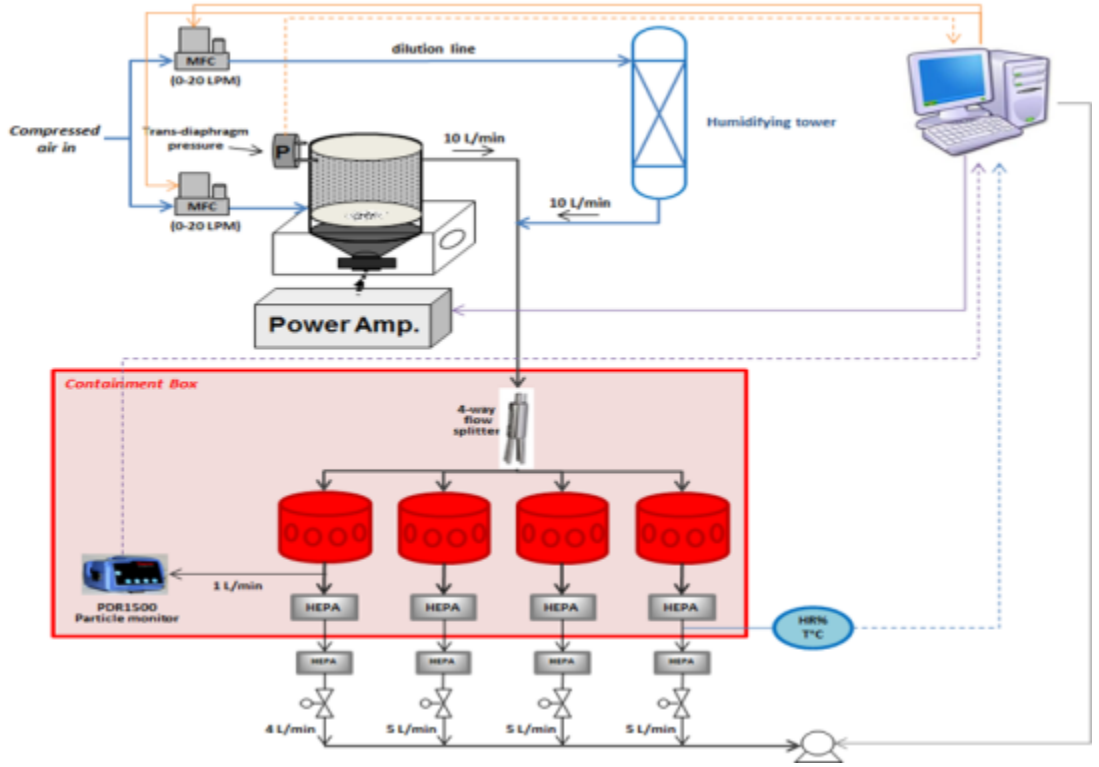
Tissue sampling

3 **30** **90** **180**

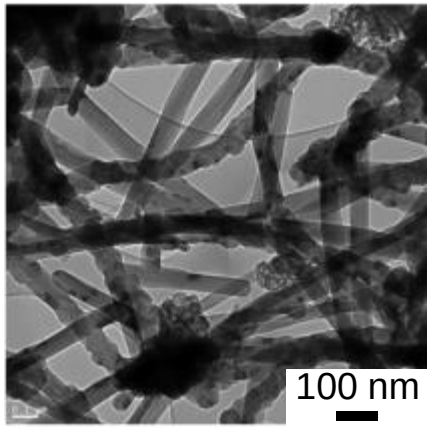
Short term **Long term**

Post exposure time (days)

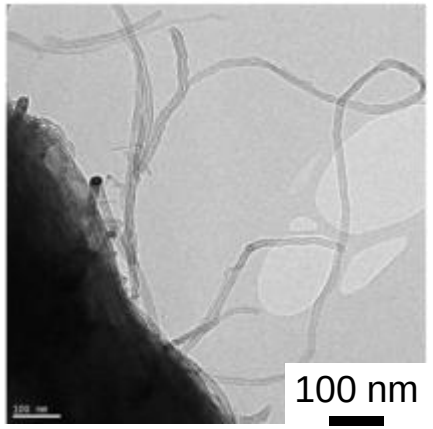
Aerosol generation



NM-401

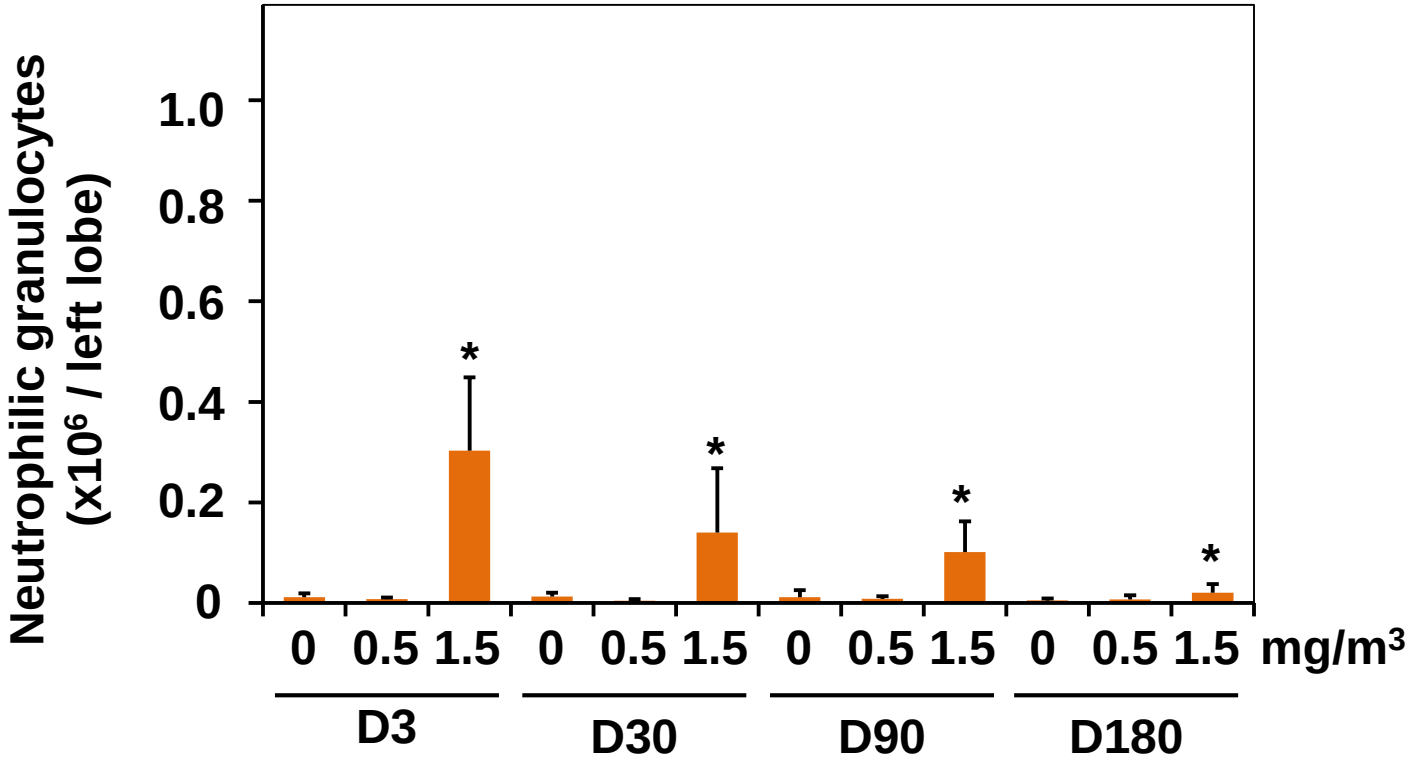


NM-403

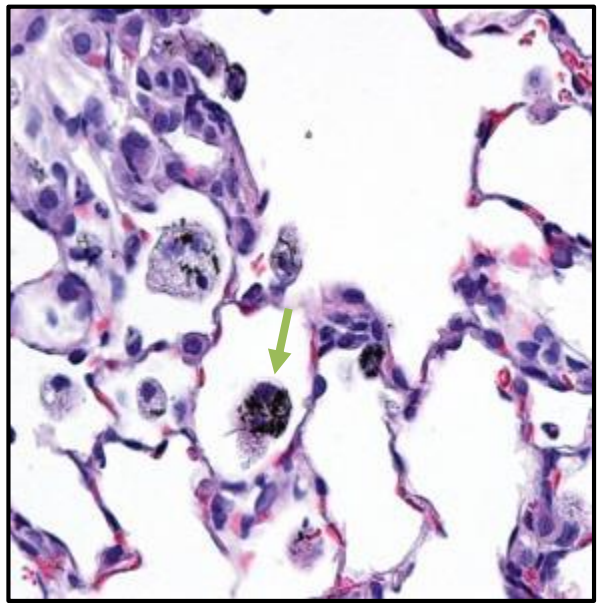


	Target concentration (mg/m^3)	Concentration (mg/m^3)	Concentration ($particles/cm^3$)	MMAD (nm)	NMAD (nm)
NM-401	0.5	0.54 ± 0.11	~815	790	280
	1.5	1.59 ± 0.24	~2200		
NM-403	0.5	0.50 ± 0.14	~130	1940	1440
	1.5	1.48 ± 0.63	~540		

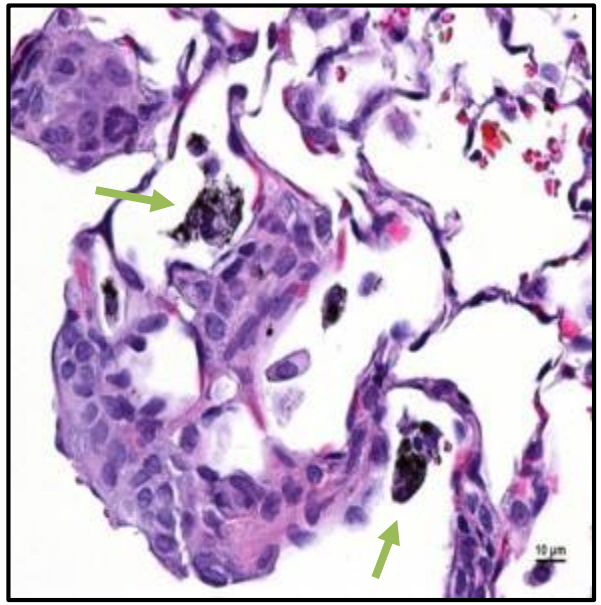
Broncho-alveolar lavage fluids cytology – *NM-401*



1.5 mg/m³



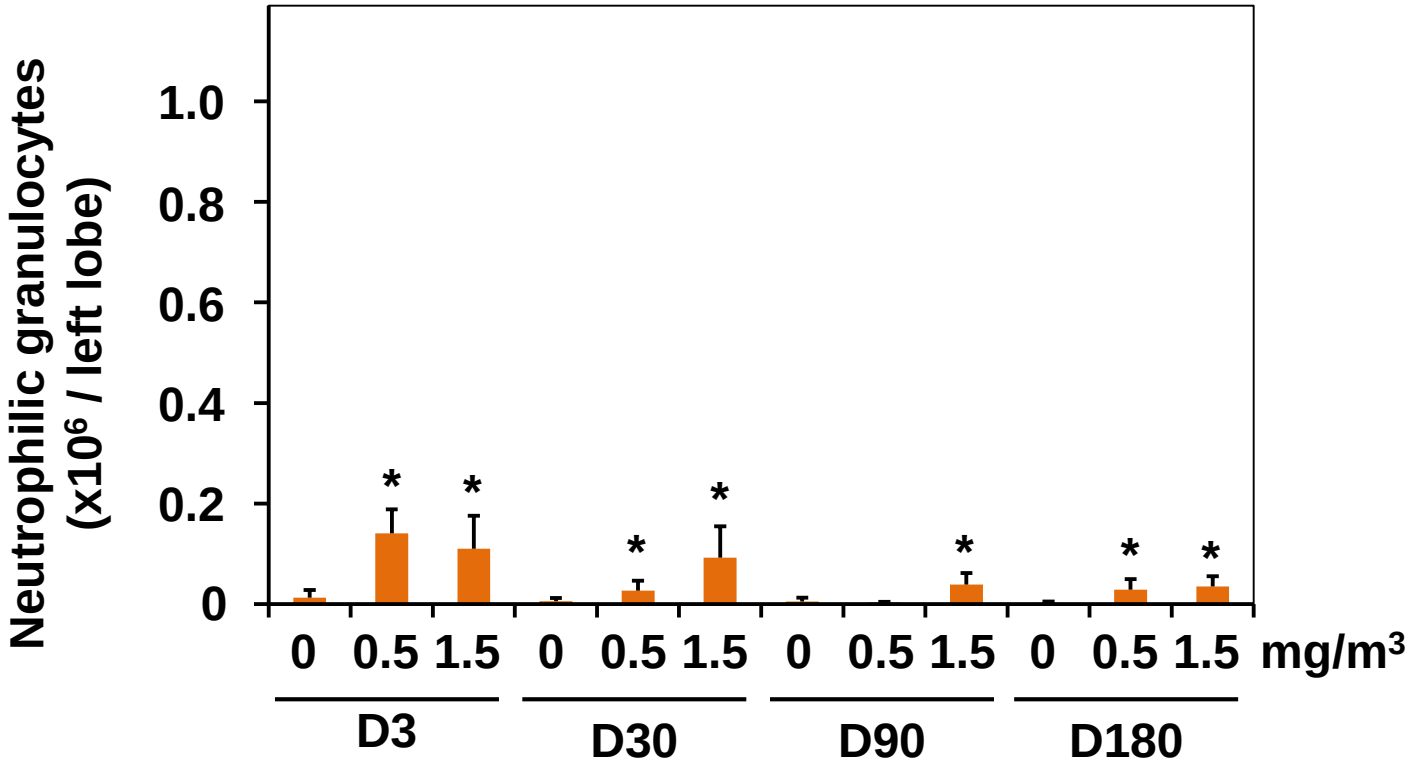
Day 3



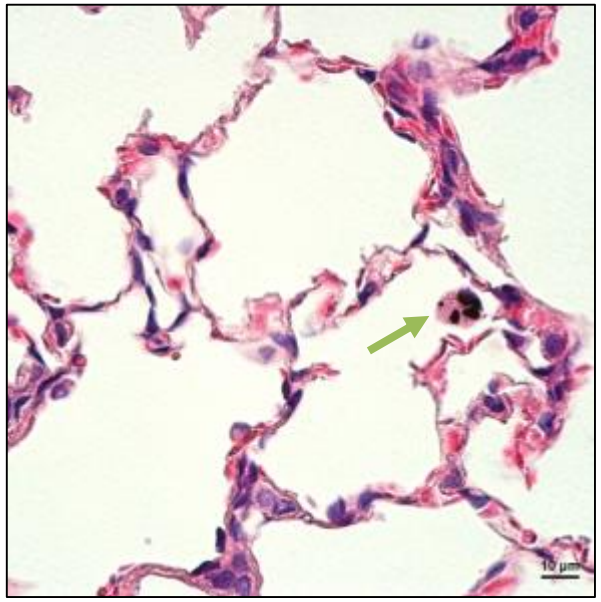
Day 180

Conventional approach

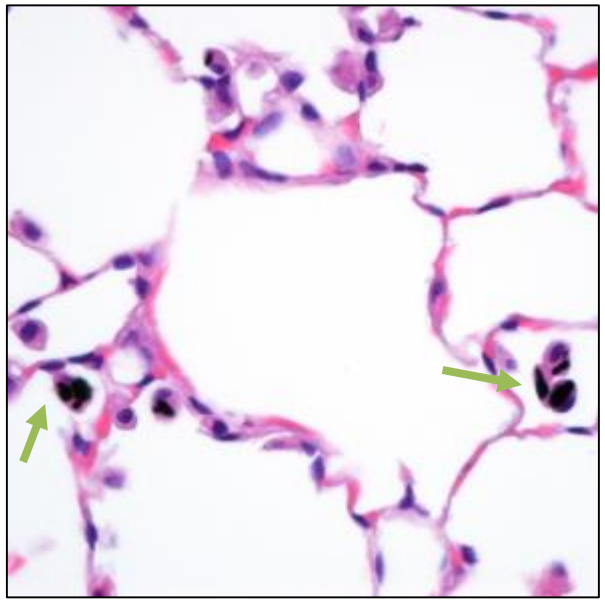
Broncho-alveolar lavage fluids cytology – *NM-403*



1.5 mg/m³



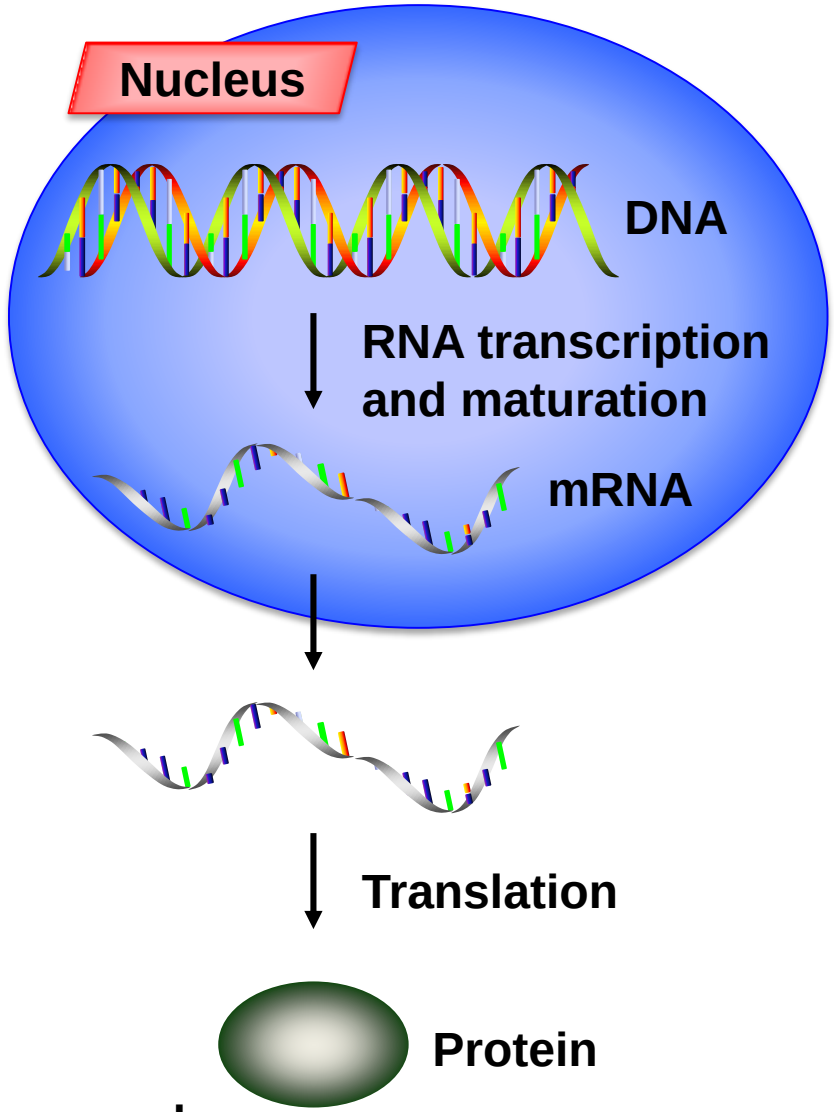
Day 3



Day 180

Conventional approach

Transcriptomic analysis (Agilent microarray)



Molecular approach

Transcriptomics =

study of the complete set of RNA transcripts produced by the genome

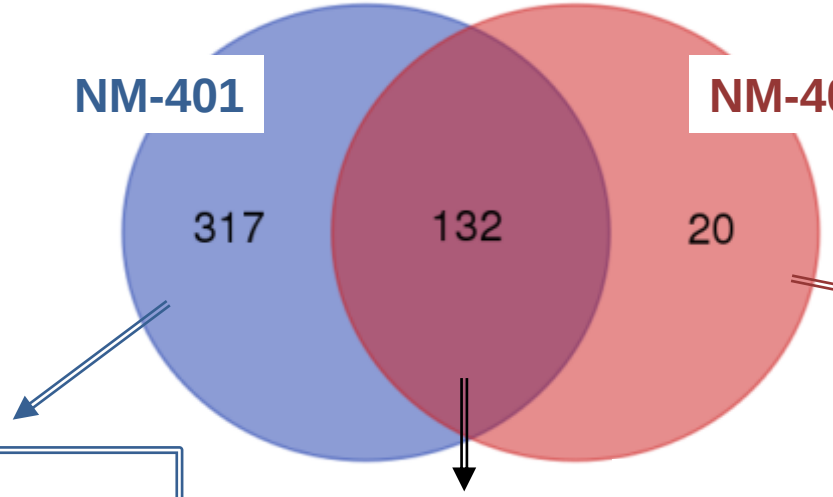
⇒ identification of gene differentially expressed in response to treatment

Microarray: Agilent technology

Data analysis: GeneSpring software

Gene clustering: DAVID database

Gene expression profile : NM-401 *versus* NM-403 (day 3)



Not enough genes for clustering

Protein folding
Response to ER stress
Positive regulation of cytokinesis
Cell division
Protein phosphorylation
Negative regulation of glucocorticoid receptor signalling pathway
Innate immune response
Positive regulation of GTPase activity

Neutrophil chemotaxis
Inflammatory response
Chemokine-mediated signaling pathway
Monocyte – lymphocyte chemotaxis
Cellular response to interleukin-1
Cellular response to interferon-γ
Cellular response to TNF
…

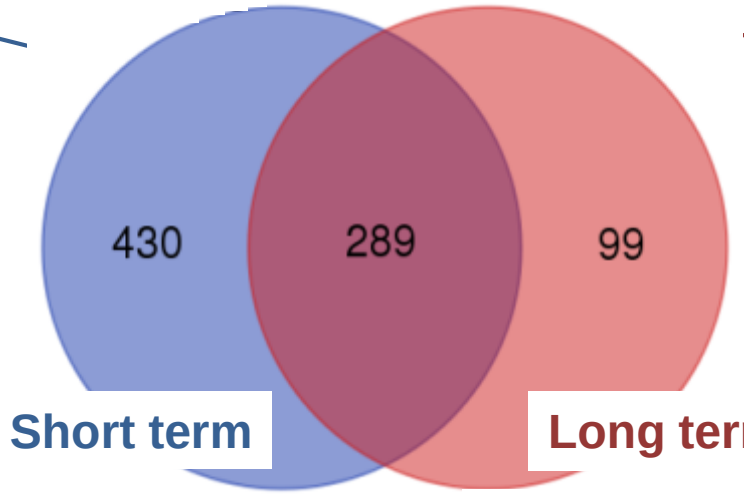
Molecular approach

GO BP: Gene Ontology Biological Processes

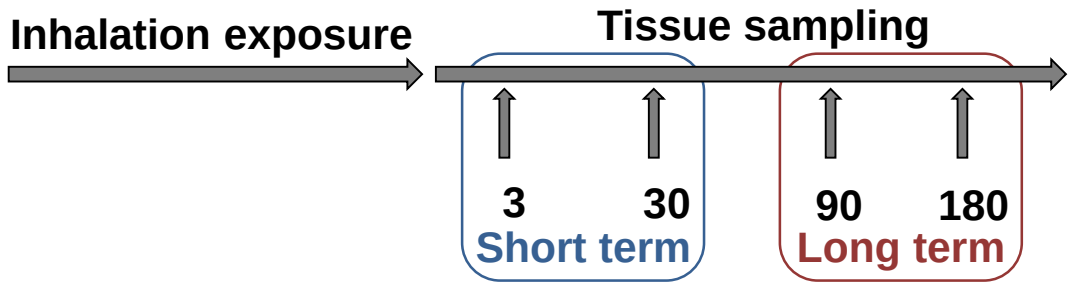
1.5 mg/m^3

Short term *versus* long term effects – NM-401

Vasodilation
Protein folding
Negative regulation of blood coagulation
Negative regulation of proteolysis
Negative regulation of endopeptidase activity
Regulation of ion transmembrane transport
Potassium ion transport



Regulation of cell shape
Chromosome segregation
Cytoskeleton organization
Mitotic cytokinesis



Molecular approach

GO BP: Gene Ontology Biological Processes

1.5 mg/m^3

Conclusions and perspectives

NM-401 *versus* NM-403 day 3

- **Both CNTs modulated expression of genes involved in the inflammatory response**
- **NM-401 : protein folding, cell division, protein phosphorylation…**
- **NM-403 : some genes not clustered**

NM-401 short *versus* long term effect

- **NM-401 modulated expression of genes involved in the inflammatory response from 3 to 180 days after the end of the exposure period even at the lower dose**
- **Short term effects : vasodilation, protein folding and ion transport**
- **Long term effects : cell cycle progression and cytoskeleton organization**

Perspectives

- **Comparison of gene expression profile in short and long term effects in NM-403 exposed lung rats**
- **Further analysis of genes specifically modulated in the long term period**

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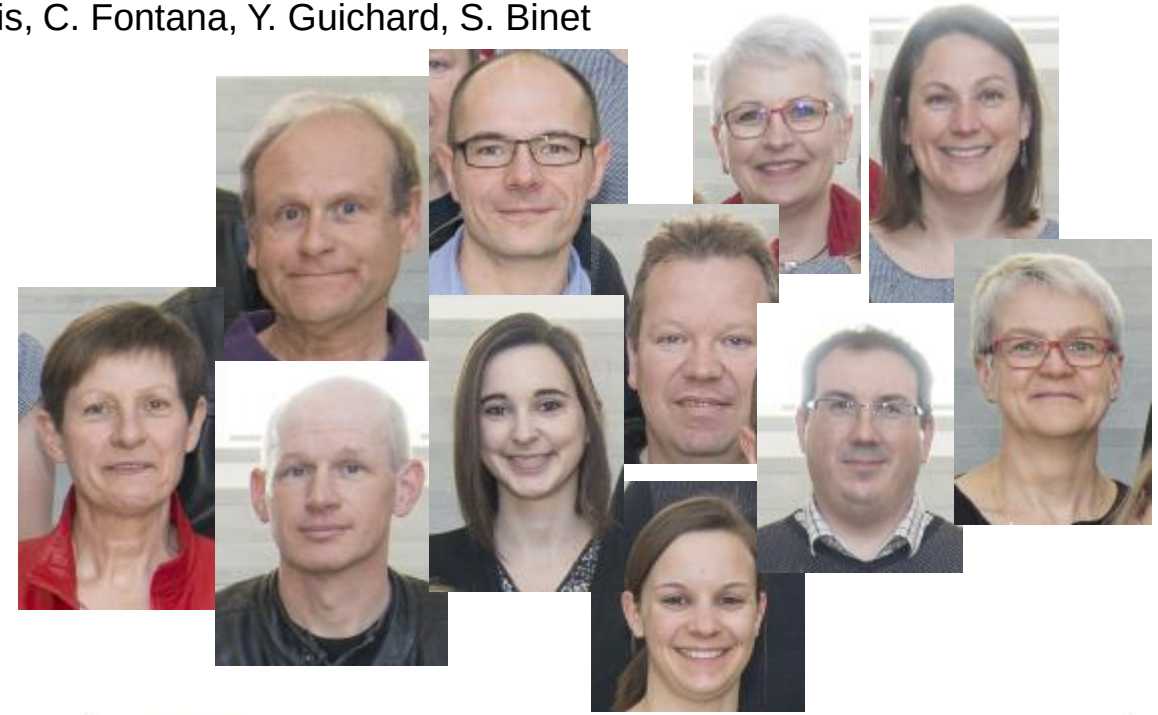

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