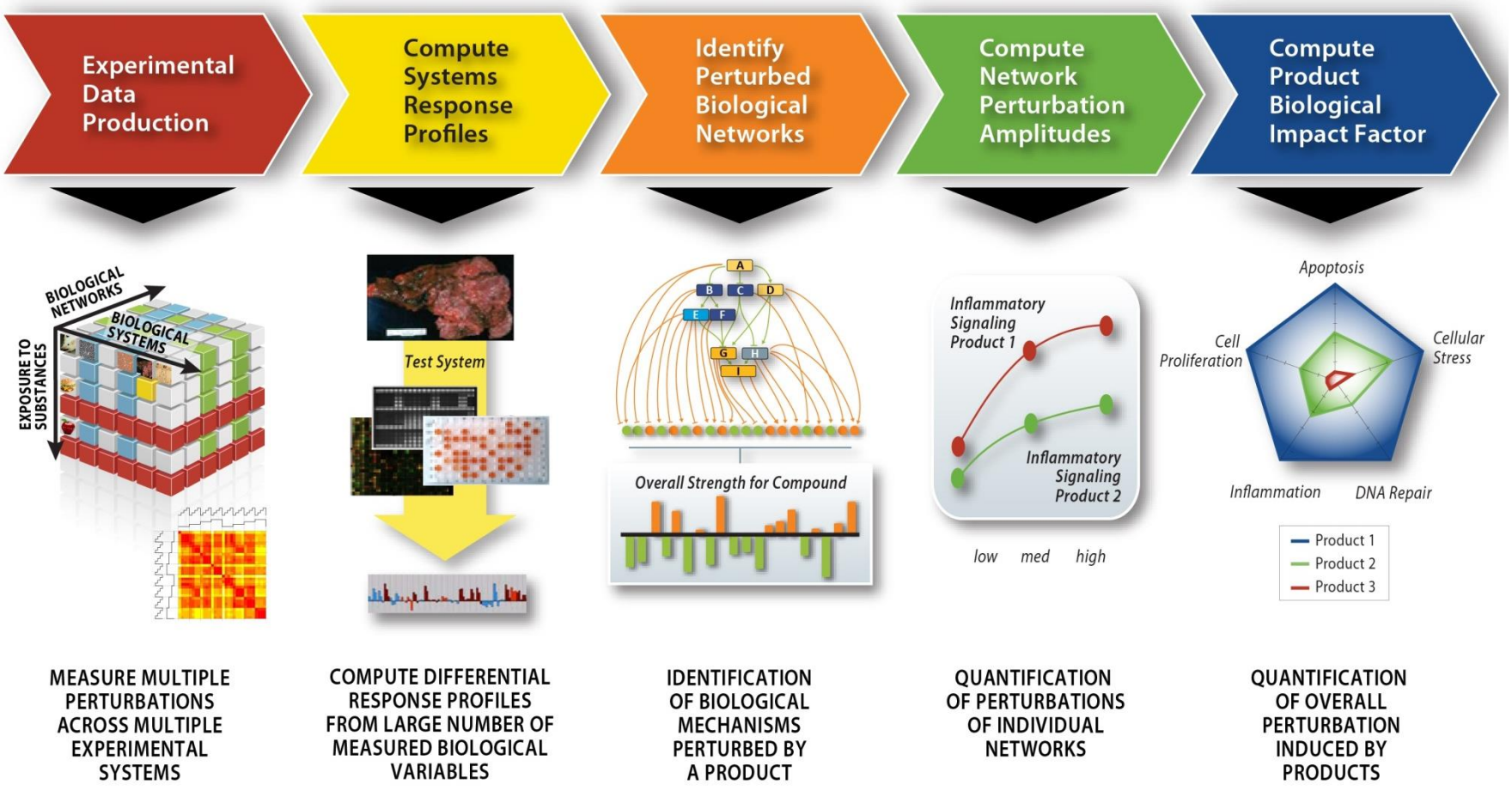
PMI RESEARCH & DEVELOPMENT

DEVELOPMENT OF A SYSTEMS TOXICOLOGY APPROACH AND ITS APPLICATION TO QUANTIFY THE BIOLOGICAL IMPACT OF TOBACCO SMOKE *IN VITRO* AND *IN VIVO*

Julia Hoeng & Manuel C. Peitsch

Philip Morris International R&D, Philip Morris Products S.A., Neuchâtel, Switzerland

Quantitative Mechanism-Based Systems Impact Assessment



Hoeng J, Deehan R, Pratt D, Martin F, Sewer A, Thomson TM, Drubin DA, Waters CA, de Graaf D, and Peitsch MC.
A network-based approach to quantifying the impact of biologically active substances. *Drug Discov Today* 17: 413-418, 2012.

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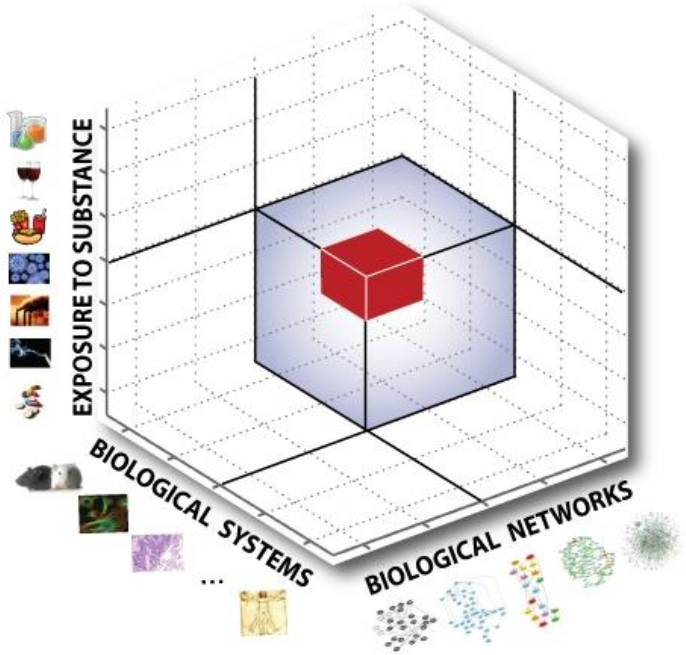
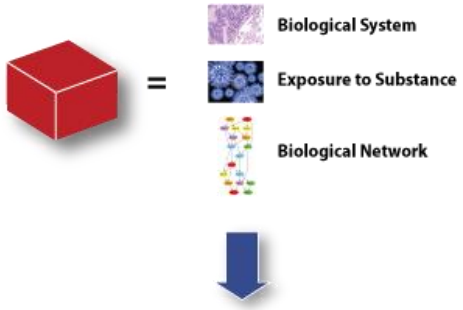
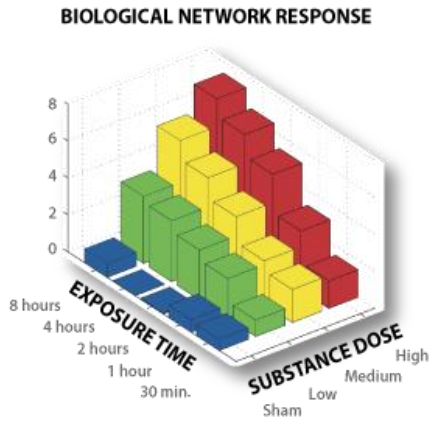
Quantitative Mechanism-Based Systems Impact Assessment

Experimental Data Production







Systematic experiments across numerous test systems

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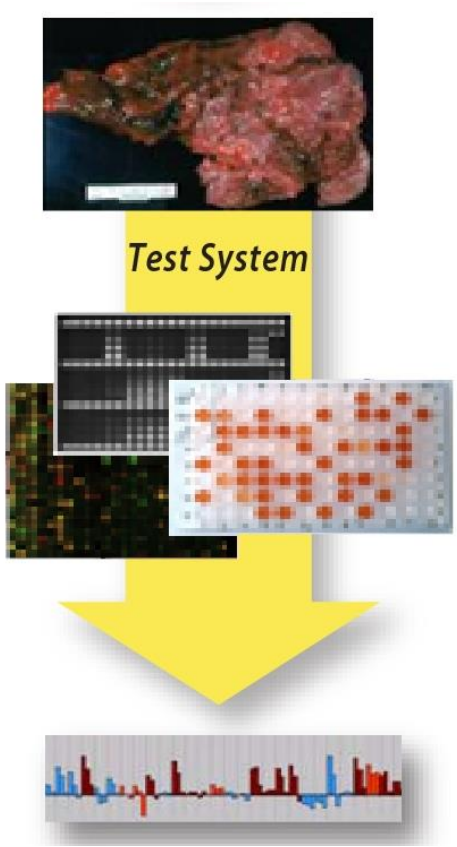
Quantitative Mechanism-Based Systems Impact Assessment

Experimental Data Production

Compute Systems Response Profiles

Compute Differential Systems Response Profiles from large numbers of measured biological variables



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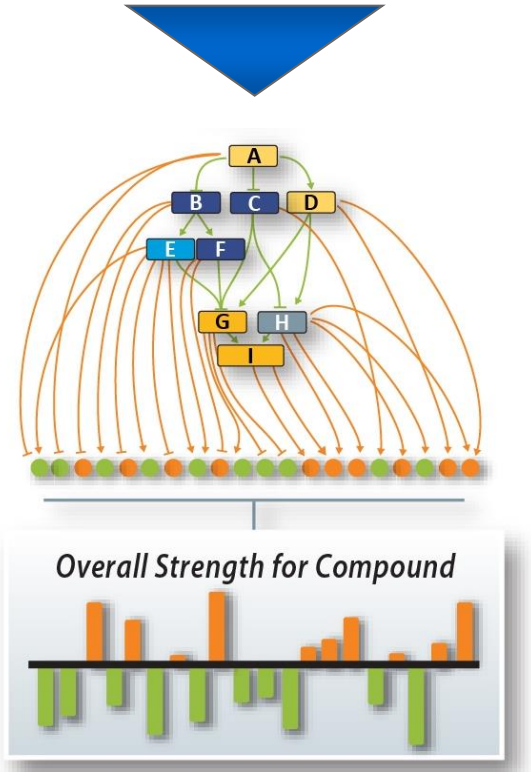
Quantitative Mechanism-Based Systems Impact Assessment

Identify biological systems that are perturbed by a product

Products



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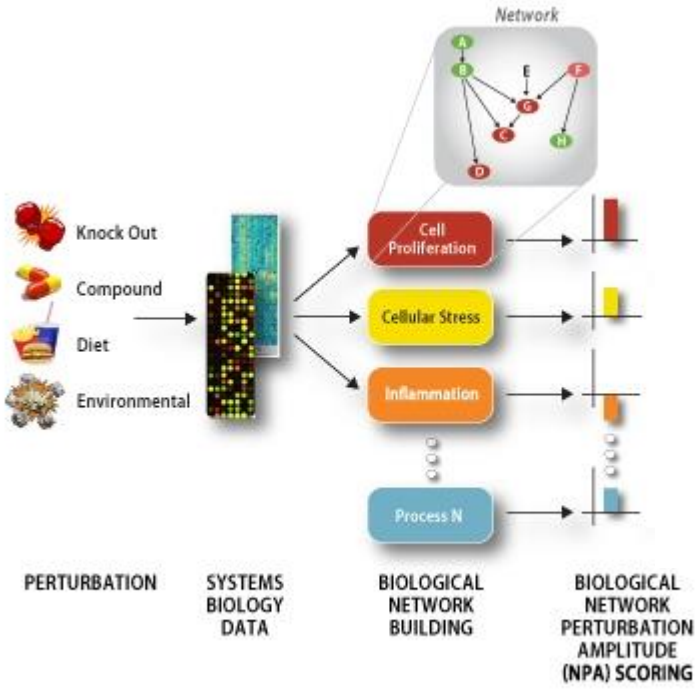
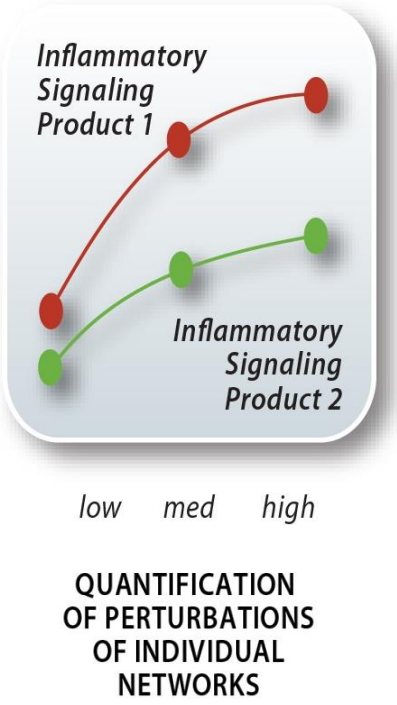
Quantitative Mechanism-Based Systems Impact Assessment

- Compute Amplitudes of Perturbation for all identified Biological Networks
- Compare the Network Perturbation Amplitudes across responses between different perturbations
- Identify potential biomarkers indicative of overall Network Perturbation State





Martin F, Thomson TM, Sewer A, Drubin DA, Mathis C, Weisensee D, Pratt D, Hoeng J, and Peitsch MC.
Assessment of network perturbation amplitude by applying high-throughput data to causal biological networks. *BMC Syst Biol* 6: 54, 2012

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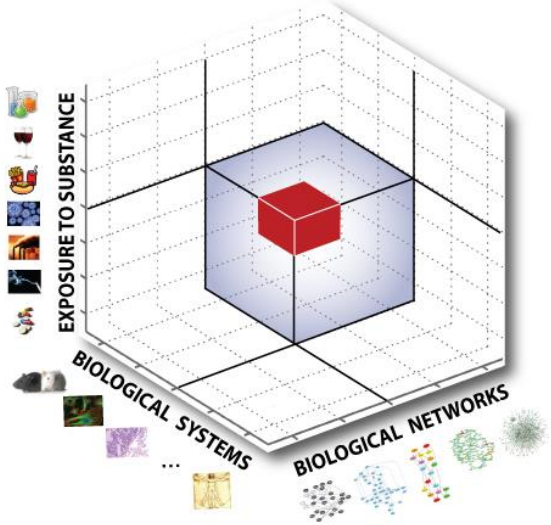
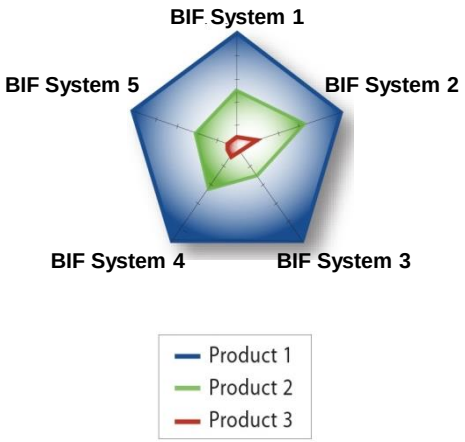
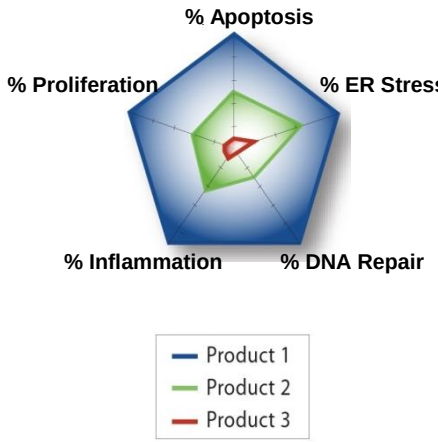
Quantitative Mechanism-Based Systems Impact Assessment

Product Biological Impact Factor is a numerical indicator of the impact of a product (a set of substances) on the system







	System 1				System 2			
Exp / BN	Prod 1	Prod 2	Prod 3	Ctrl	Prod 1	Prod 2	Prod 3	Ctrl
Apoptosis	10	3	2	0	5	4	3	1
Oxidative Stress	5	4	4	1	4	2	2	0
Proliferation								
.....								
.....								

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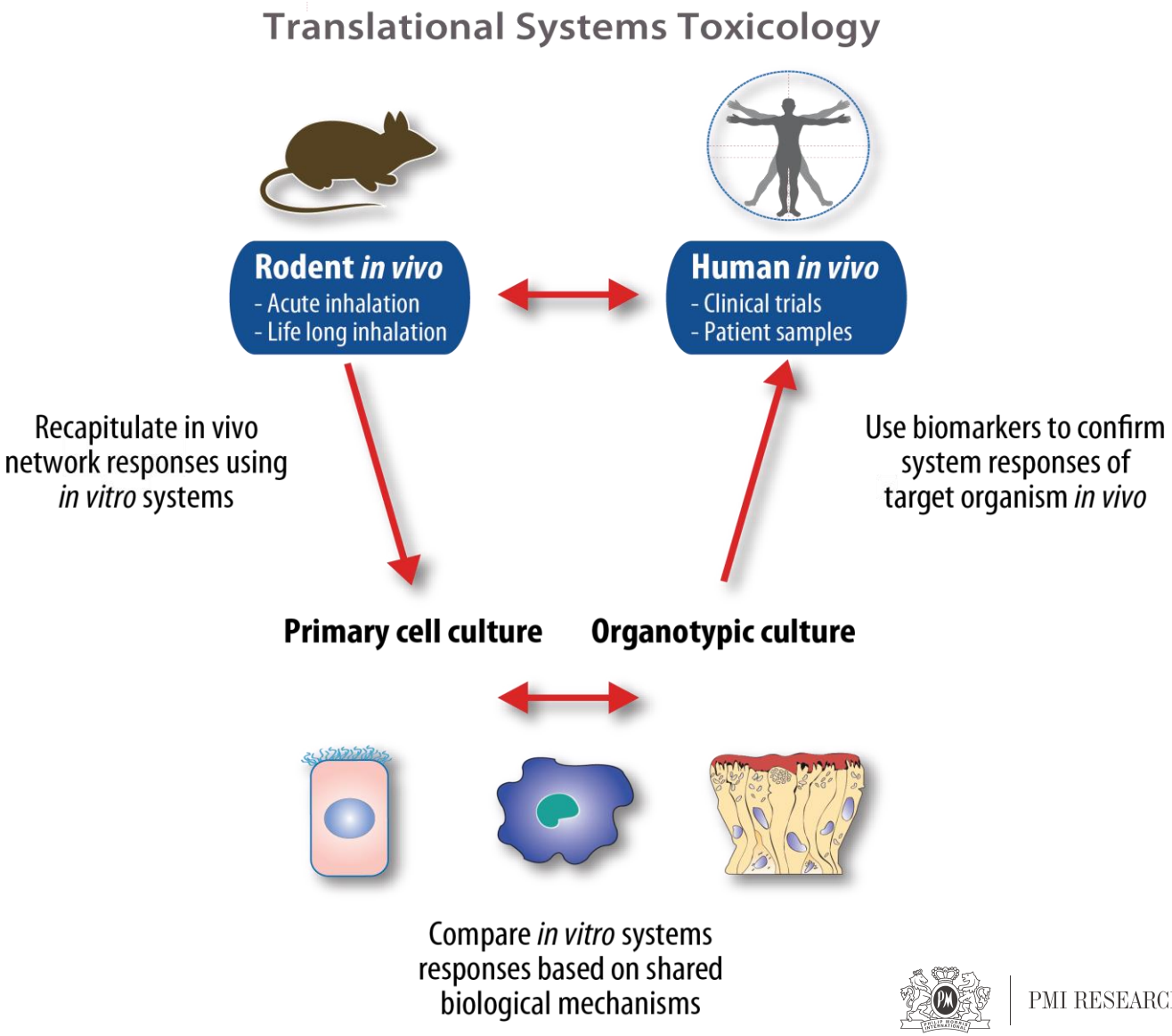
Translational Systems Toxicology



PMI RESEARCH & DEVELOPMENT

Establishment of the rat counterpart of the human organotypic bronchial tissue

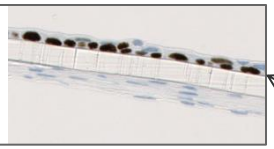
Isolation amplification seeding of rat primary cells

Trachea
Bronchi

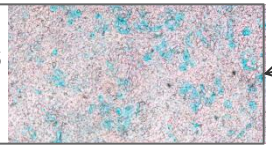
Air-liquid interface & differentiation

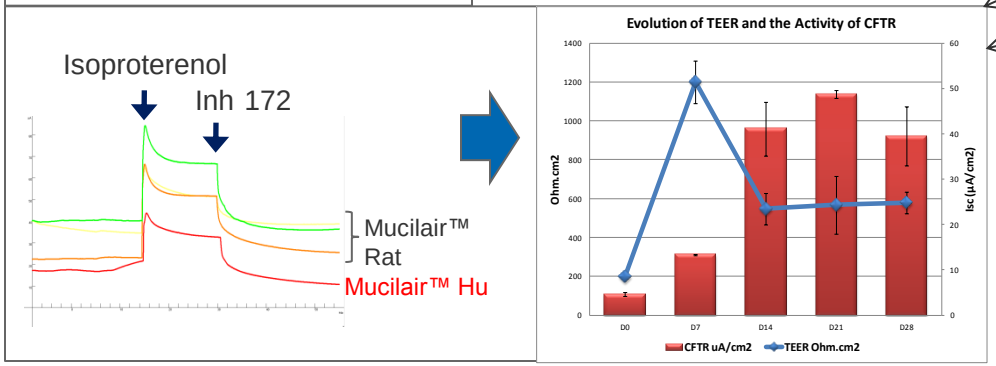
Ion channel activities:
Isoproterenol: a non-selective beta adrenergic agonist increases the intra-cellular cAMP and activates ion channels such as CFTR.
Inh 172: Specific inhibitor of CFTR.

p63 staining (basal cells)



12 % of mucus releasing cells





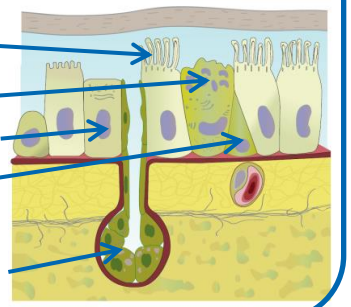
Main Characteristics:

- ✓Differentiation in basal cells
- ✓ Presence of mucus
- ✓ Presence of tight junctions (TEER)
- ✓ Active ion channels (e.g. CFTR)
- ✓Air-Liquid Interface Model
- ✓ Made of primary rat cells (epithelial cells and fibroblasts)

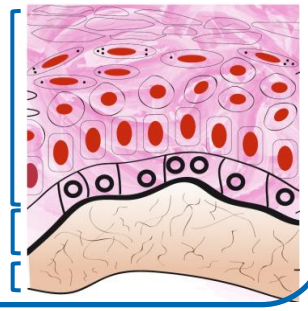
PMI RESEARCH & DEVELOPMENT

Recapitulation of *In Vivo* Biology by Organotypic Systems

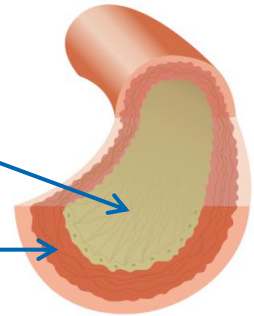
Nasal epithelium

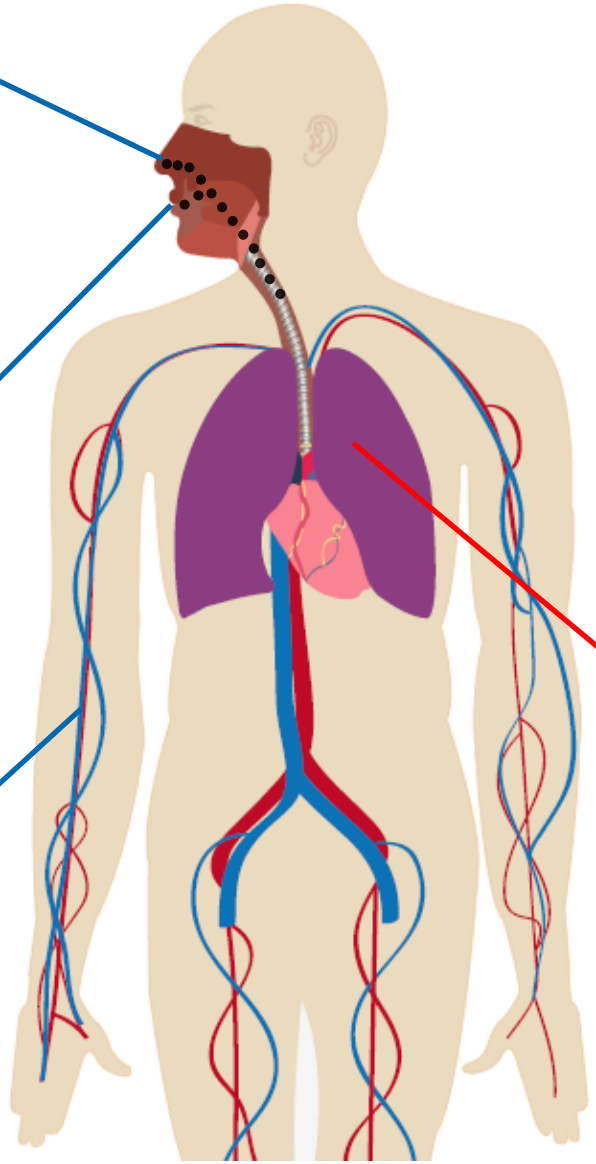


Buccal and Gingival Mucosa

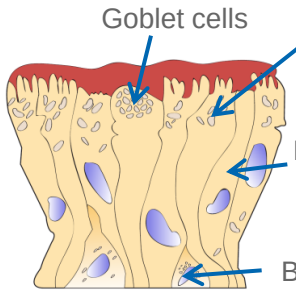


Blood vessel





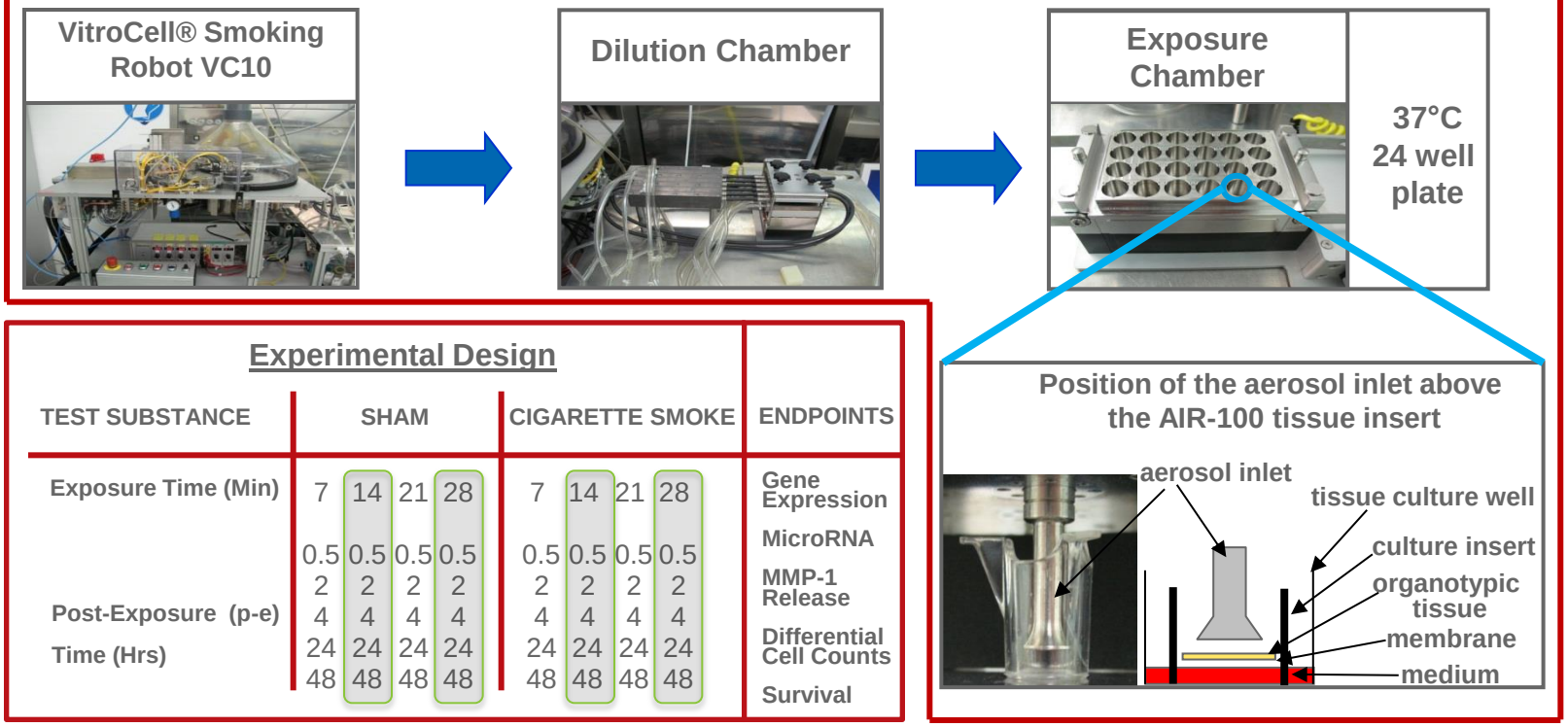
Bronchial Epithelium



PMI RESEARCH & DEVELOPMENT

Organotypic Cultures of Human Primary Bronchial Epithelial Cells Exposed to Whole Cigarette Smoke (CS)

Primary Organotypic Culture of Human Bronchial Epithelial Cells Exposed to Whole CS



Experimental Design

TEST SUBSTANCE	SHAM				CIGARETTE SMOKE				ENDPOINTS
Exposure Time (Min)	7	14	21	28	7	14	21	28	Gene Expression
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	MicroRNA
	2	2	2	2	2	2	2	2	MMP-1 Release
Post-Exposure (p-e)	4	4	4	4	4	4	4	4	
Time (Hrs)	24	24	24	24	24	24	24	24	Differential Cell Counts
	48	48	48	48	48	48	48	48	Survival

Position of the aerosol inlet above the AIR-100 tissue insert

Experimental Data Production

Upper panel: Organotypic cultures of human primary bronchial epithelial cells were directly exposed to mainstream CS using the Vitrocell® system. Lower panel: The cells were exposed to CS during four different exposure times, then various endpoints were captured after different post-exposure times.

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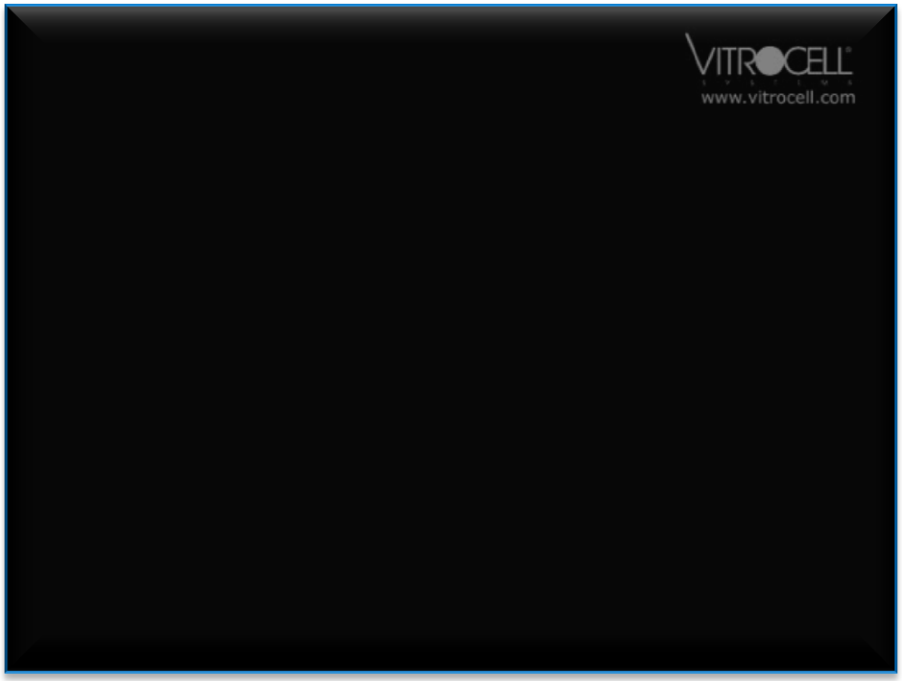
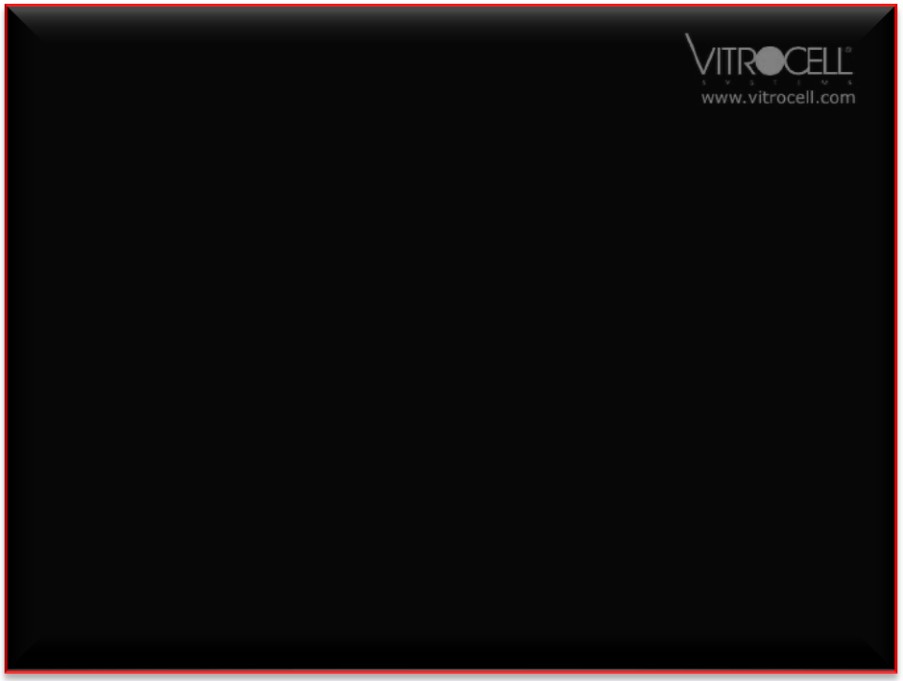
MATTEK STUDY

Whole cigarette smoke/aerosol exposure system (Vitrocell®)

VITROCELL® EXPOSURE SYSTEM



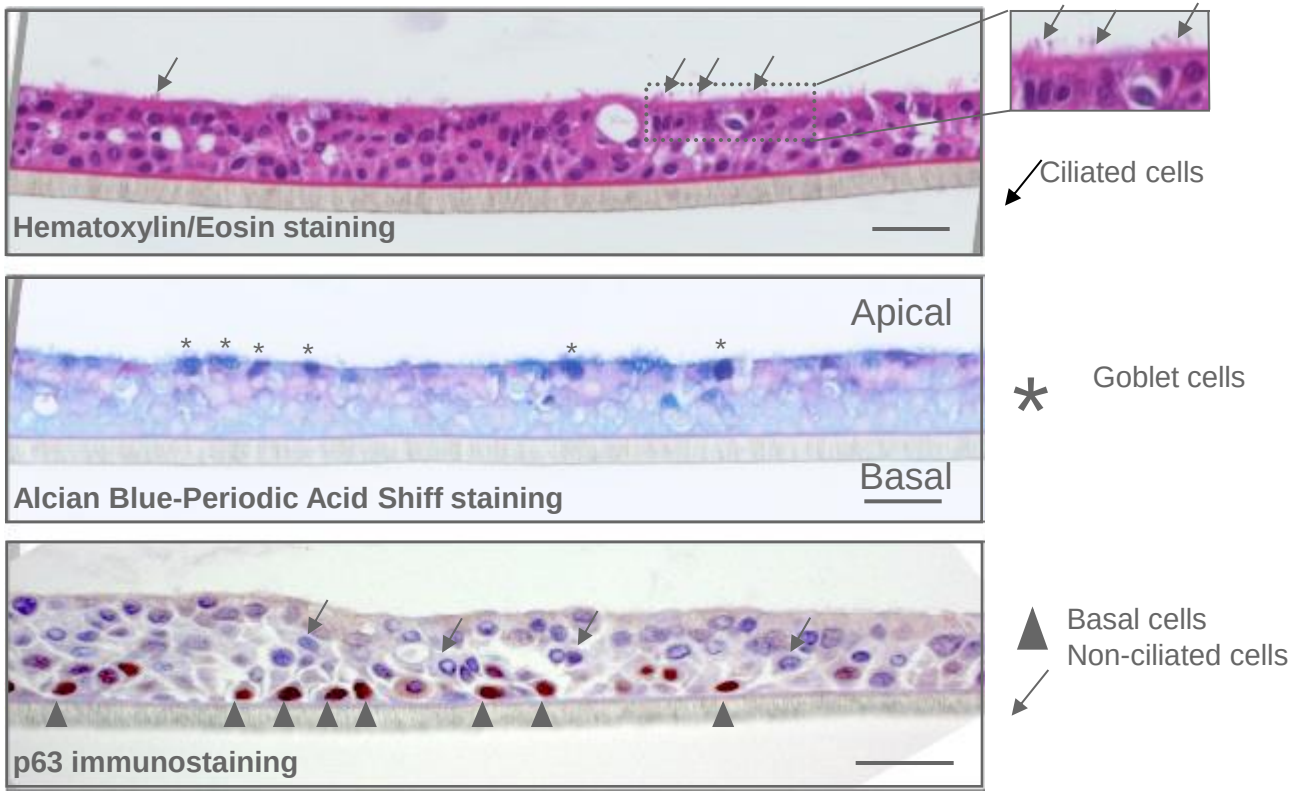
VITROCELL® DEPOSITION SENSOR



MOVIES

PMI RESEARCH & DEVELOPMENT

Human organotypic bronchial epithelial cells exposed to CS resemble bronchial epithelium from human smokers



Unperturbed human organotypic bronchial epithelial cell culture resembles closely to human lung epithelium both at the morphological level (Karp et al. 2002) and at the molecular level (Pezzulo et al. 2011).

Human organotypic bronchial epithelial cells exposed to CS resemble bronchial epithelium from human smokers

- For all four *in vivo* smoking gene signatures used in the GSEA, a similar pattern of enrichment score was found in CS-exposed AIR-100 up-regulated gene regulation profile (Fig. A) and in down-regulated gene regulation profile (Fig. B)
- Com… …o and *in vitro*
- Gene… …le genes after… …gulated gene…

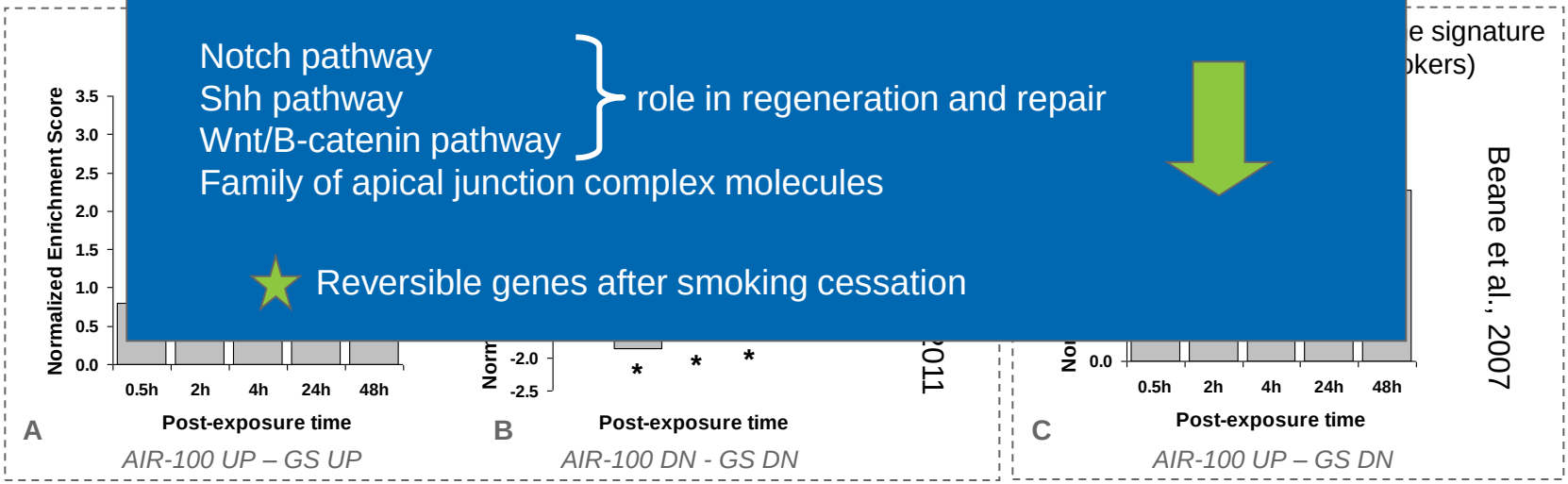
Functional Analysis

In both *in vivo* / *in vitro* context

Metabolism of xenobiotics ★
Redox balance ★
Glutathione metabolism
Pentose phosphate pathway ★
Many direct target genes of Nrf2 transcription factor

Notch pathway
Shh pathway
Wnt/B-catenin pathway
} role in regeneration and repair
Family of apical junction complex molecules

★ Reversible genes after smoking cessation

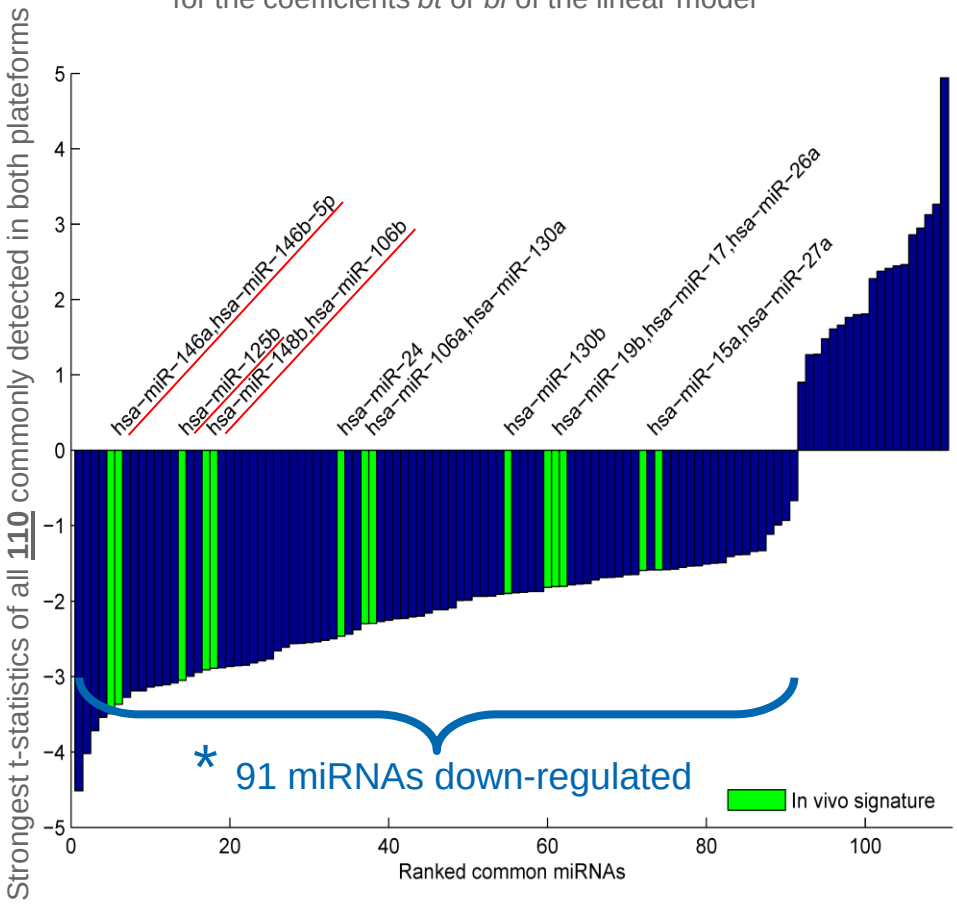


Human organotypic bronchial epithelial cells exposed to CS resemble bronchial epithelium from human smokers

- Only one human *in vivo* miRNA from bronchial epithelial cells published so far (Schembri et al. 2009).
- Out of ~ 230 miRNAs detectable in this tissue context, half of them are commonly detected in both studies. Only 14 miRNAs differentially expressed are common between both *in vivo* and *in vitro* datasets (GREEN tag).
- CS down-regulates a large majority of miRNA expression (*: 91 miRNAs out of 110) in both *in vivo* and *in vitro* situation.
- The biological functions associated with some of the highly "translatable" miRNAs are related to inflammation (miR-146b and miR-125b) and cell cycle processes (miR-106a and miR-106b) that are also known to be perturbed by CS in lung tissue context.

Comparison between *in vivo* human smoker miRNA signature and CS-exposed AIR-100 *in vitro* miRNA dataset.

The vertical axis of the bar plot represents the best t-statistics (i.e. lowest negative or highest positive t-scores) obtained for the coefficients *bt* or *bi* of the linear model



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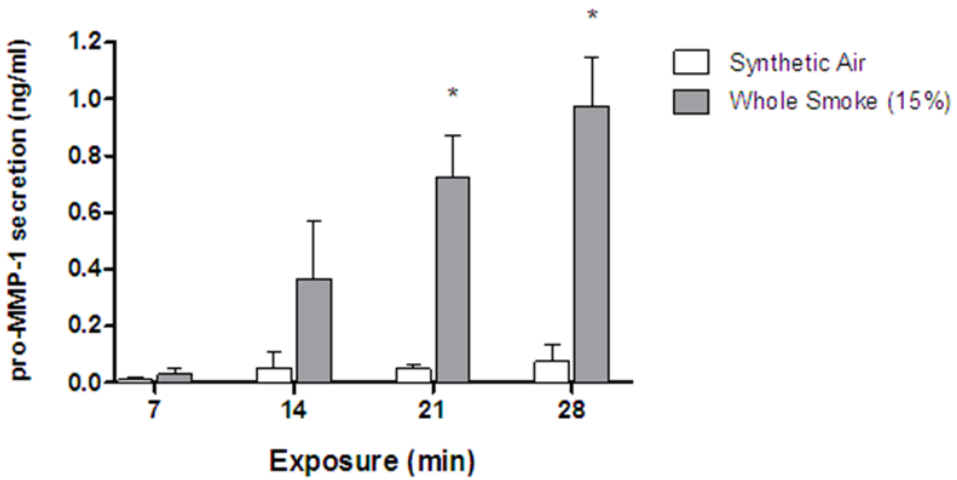
IN VITRO / IN VIVO COMPARISON – MATTEK STUDY

Human organotypic bronchial epithelial cells exposed to CS resemble bronchial epithelium from human smokers

BACKGROUND

- MMP-1 is an interstitial collagenase involved in tissue remodeling and repair during lung development and inflammation.
- MMP-1 is known to be up-regulated upon CS exposure both *in vivo* and *in vitro* (Mercer et al. 2004, Lahmann et al. 2001, Philips et al. 2005).
- Human MMP-1 promoter contains CS-regulatory elements (Mercer et al. 2009).



48 hours post-exposure time
Detection of pro-MMP-1 protein in the AIR-100 culture medium via ELISA assay

Human bronchial epithelial cells cultured at the air-liquid interface respond to CS exposure by releasing higher level of pro-MMP-1 as seen *in vivo* in smoker's tissue.

PMI RESEARCH & DEVELOPMENT

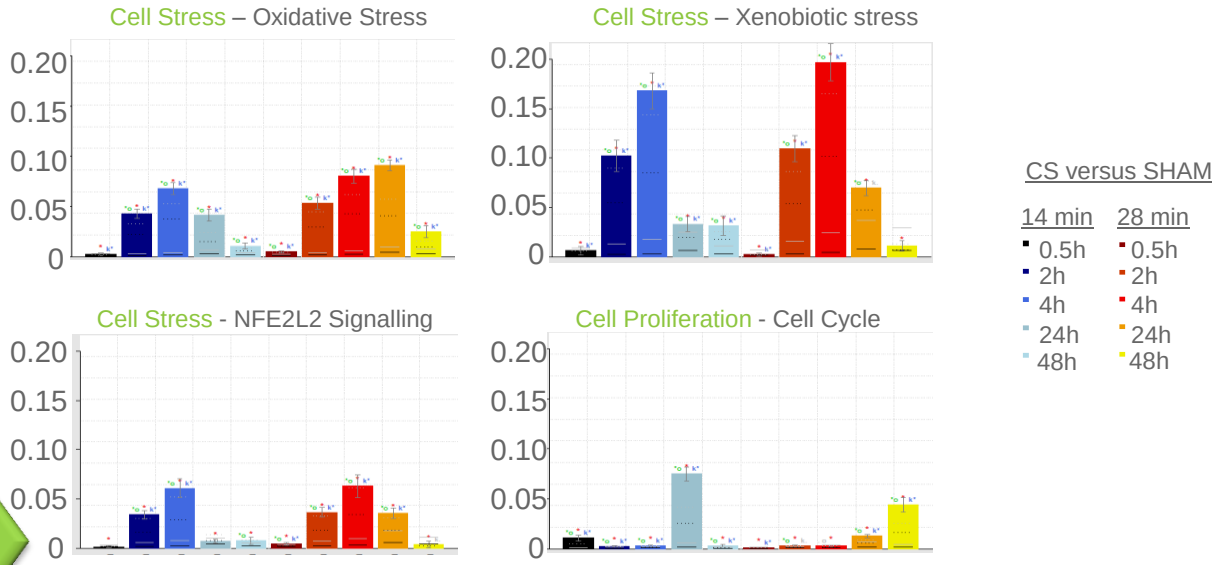
Human organotypic bronchial epithelial cells exposed to CS resemble bronchial epithelium from human smokers

- Many of the biological functions known to be directly affected upon CS exposure, both *in vivo* and *in vitro*, were identified based on the functional analysis of the leading edges genes that participate to the highest enrichment score observed at 4 hours post-exposure.
- A single exposure to CS induces a similar biological perturbation (at the level of gene expression, miRNAs expression or MMP-1 secretion) in an *in vitro* human organotypic bronchial epithelium-like tissue culture to the one observed *in vivo* in the airway epithelium of human smokers.

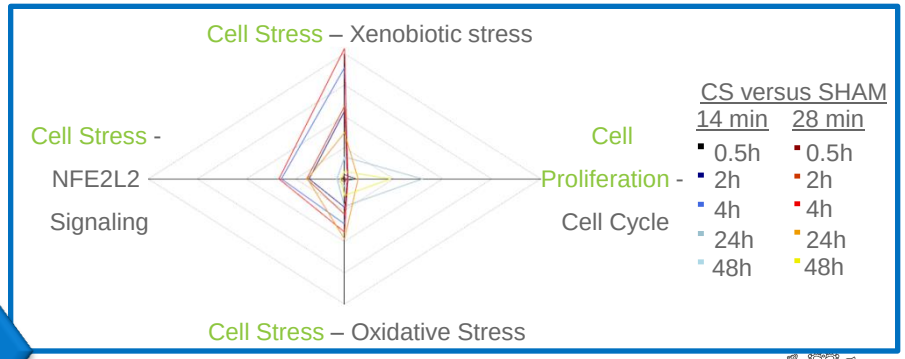
"Human bronchial epithelial cells exposed in vitro to cigarette smoke at the air-liquid interface resemble bronchial epithelium from human smokers."

Am J Physiol Lung Cell Mol Physiol. 2013 Apr;304(7):L489-503

Mechanistic Investigation with NPA and BIF



Compute Network Perturbation Amplitudes



Compute Product Biological Impact Factor

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Xenobiotic Metabolism Network

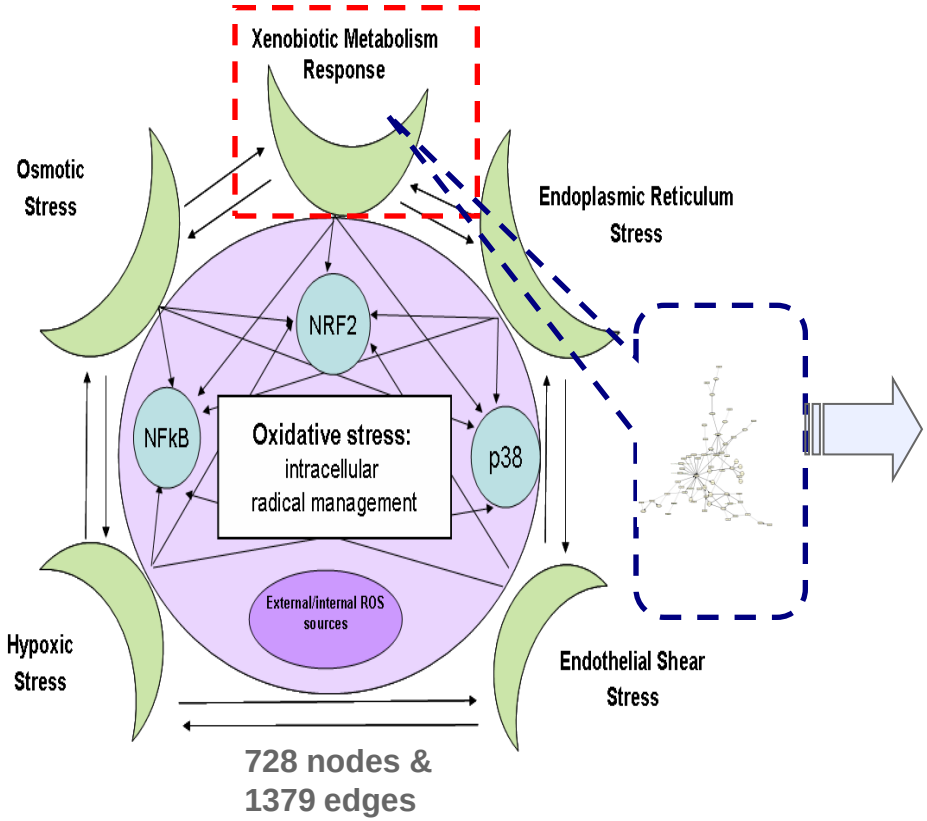
The xenobiotic metabolism (XM) network model for rat, mouse, and human respectively, which is a sub-network of Cellular Stress Network model, was built by PMI scientists in collaboration with Selventa, Cambridge, MA.

Network Definition

Boundary Overview

- Focus on cell types found in **non-diseased pulmonary** and **cardiovascular** systems
- Focus on **acute response** to **physiological stressors**
- Includes areas of **biology** known to be **modulated by smoke**

Ensure network met the research needs of PMI



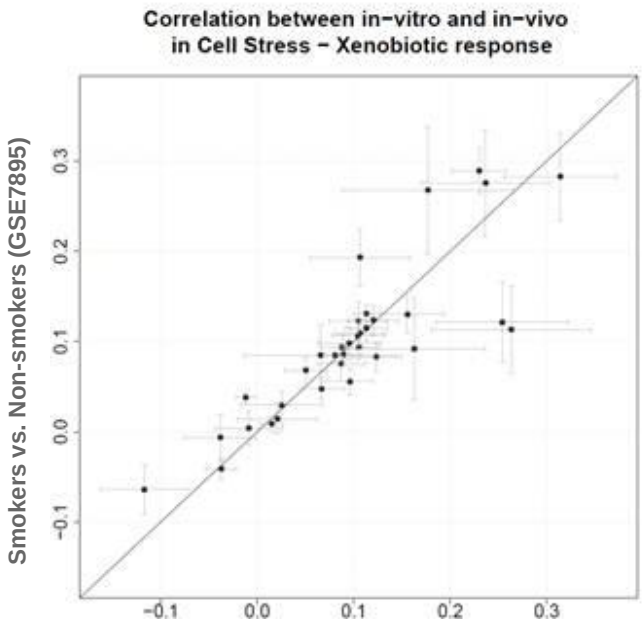
Schlage, W., J. Westra, et al. (2011). "A computable cellular stress network model for non-diseased pulmonary and cardiovascular tissue." BMC Systems Biology **5**(1): 168.

PMI RESEARCH & DEVELOPMENT

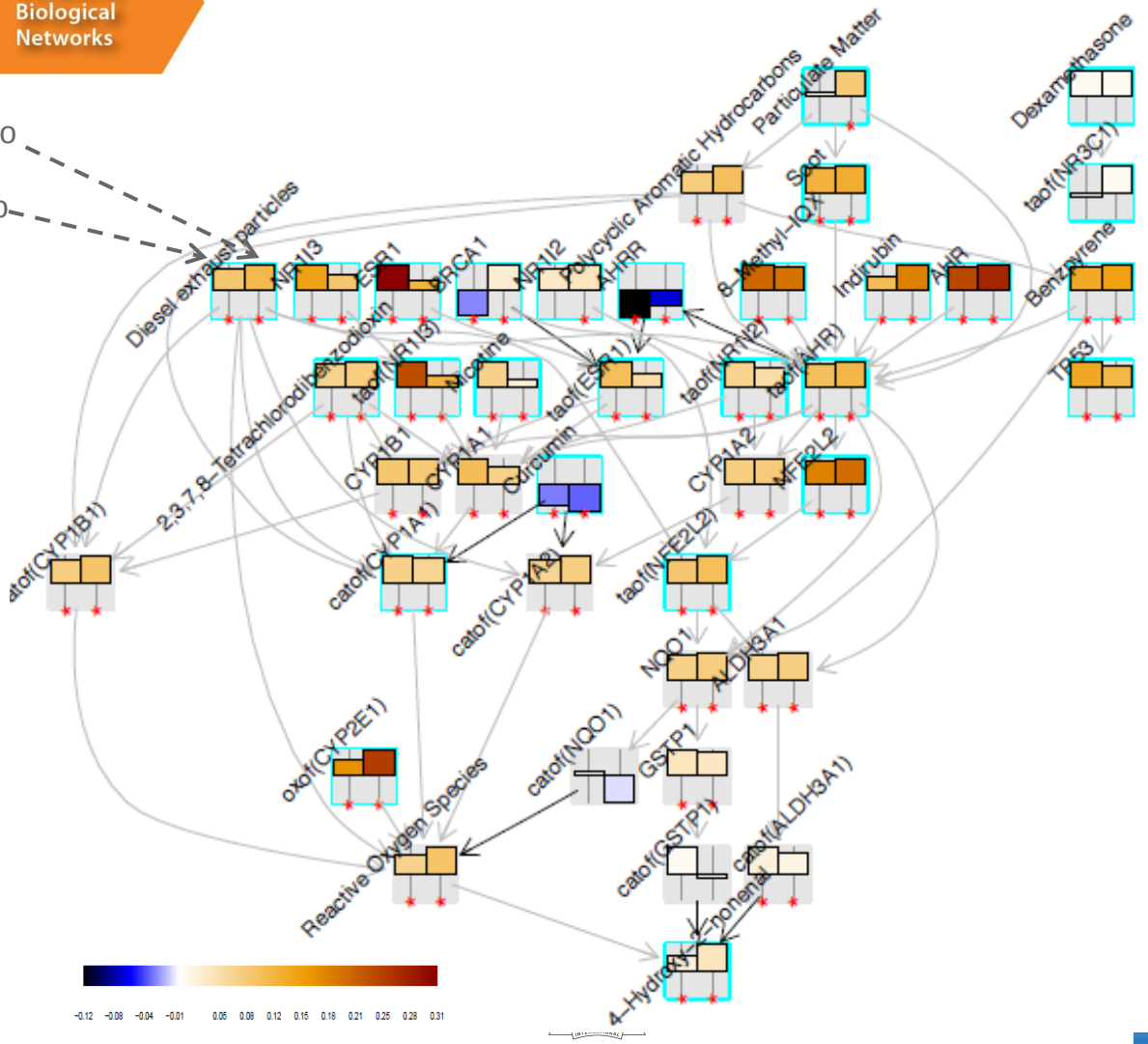
Activation of Xenobiotic Metabolism in Human and Organotypic Airway Culture Exposed to Whole Smoke

Experimental Data Production → Compute Systems Response Profiles → Identify Perturbed Biological Networks

- Right In vivo
- Left: In vitro

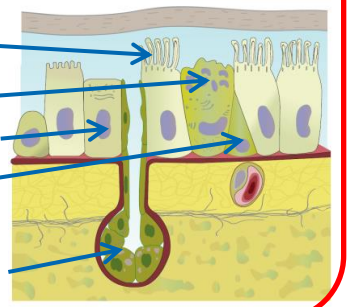


Organotypic Airway Culture Exposed to Whole Smoke vs. Sham

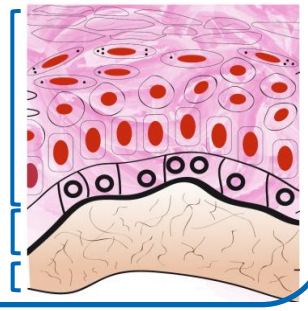


Recapitulation of In Vivo Biology by Organotypic Systems

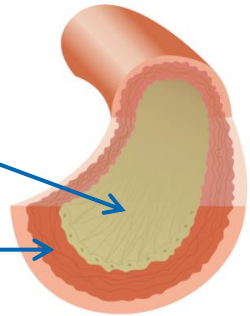
Nasal epithelium

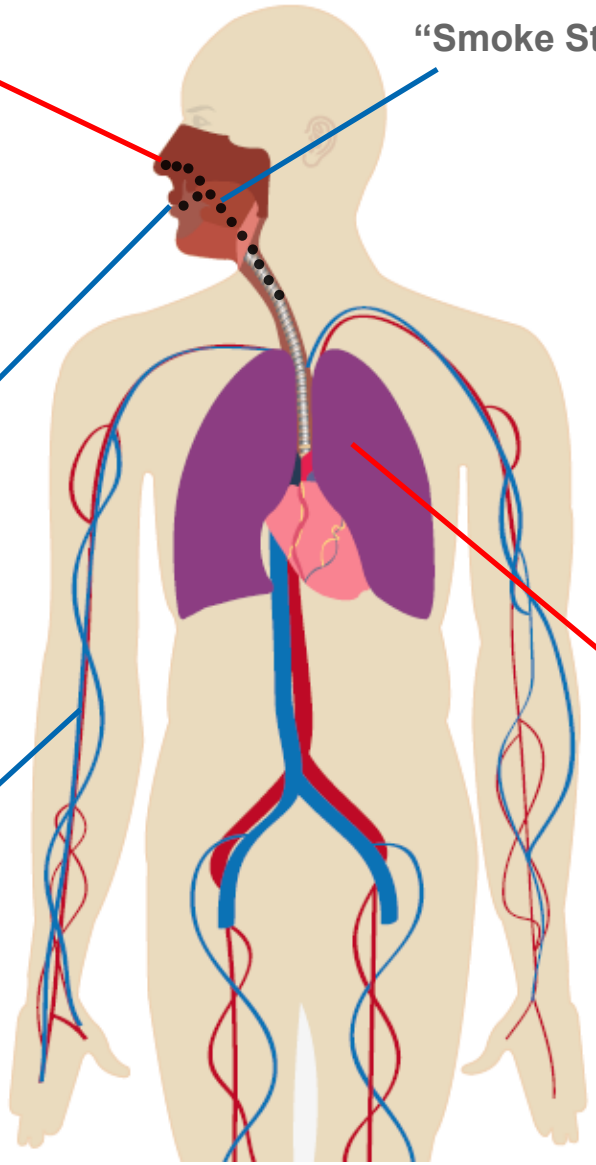


Buccal and Gingival Mucosa

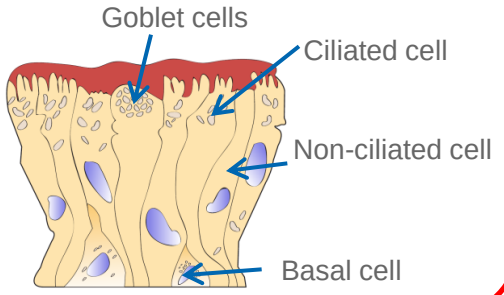


Blood vessel





Bronchial Epithelium

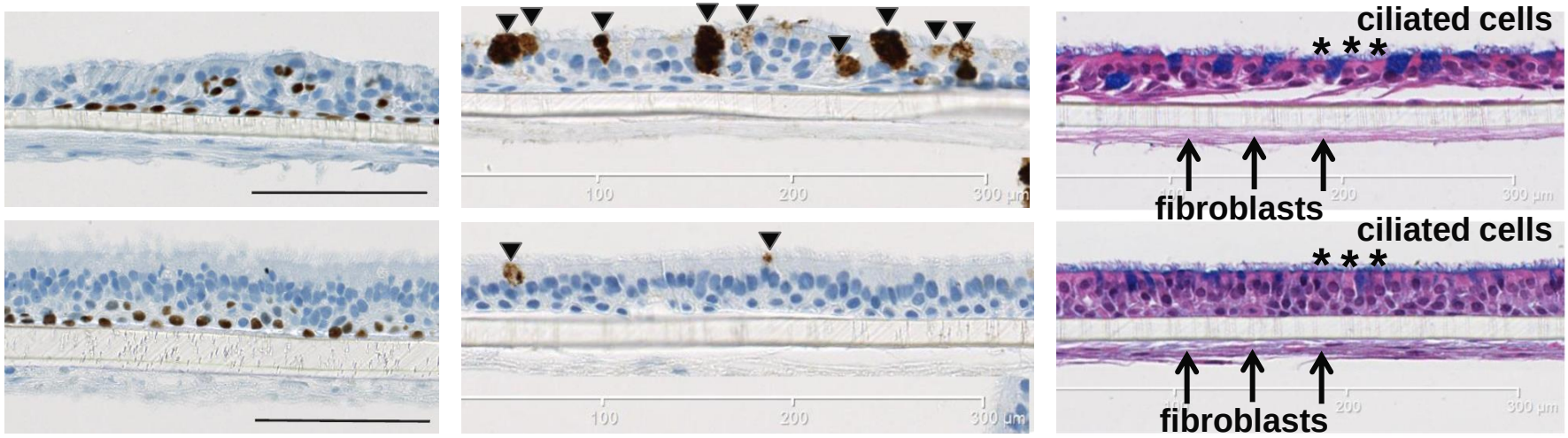


PMI RESEARCH & DEVELOPMENT

Human Organotypic Cultures of Primary Bronchial and Nasal Epithelial Cells

COMPARISON AT THE MORPHOLOGICAL LEVEL

(ANALYSIS DONE ON UNTREATED TISSUES)



p63⁺ basal cells

Muc5AC⁺ mucus secreting cells

Hematoxylin/Eosin

SMOKE STREET STUDY

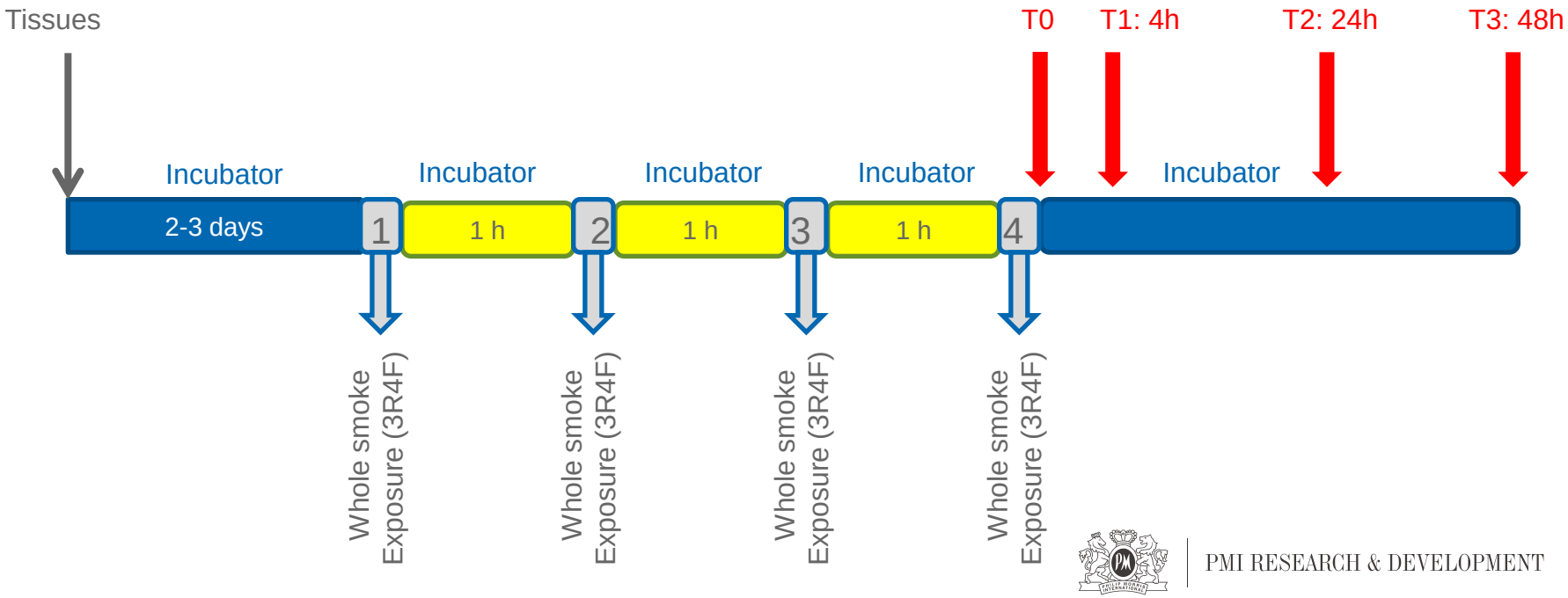
PMI RESEARCH & DEVELOPMENT

Whole Smoke Repeated Exposure of Organotypic Cultures of Human Primary Bronchial and Nasal Epithelial Cells

Experimental Data Production

Experimental Design

HUMAN TISSUES	CONDITIONS	ENDPOINTS	POST-EXPOSURE TIME 0h	4h	24h	48h
BRONCHIAL MUCILAIR™ NASAL MUCILAIR™	SHAM CS 10% CS 16%	Cytotoxicity (LDH assay)			X	X
		Membrane Integrity (TEER)			X	X
		Gene Expression Profiles (Affymetrix)	X	X	X	X
		MicroRNA Expression Profiles (GeneChip)	X	X	X	X
		Histology (Alcian blue, H&E)			X	
		Immunohistology (p63, Ki67, B-Tubulin, Muc5AC)			X	



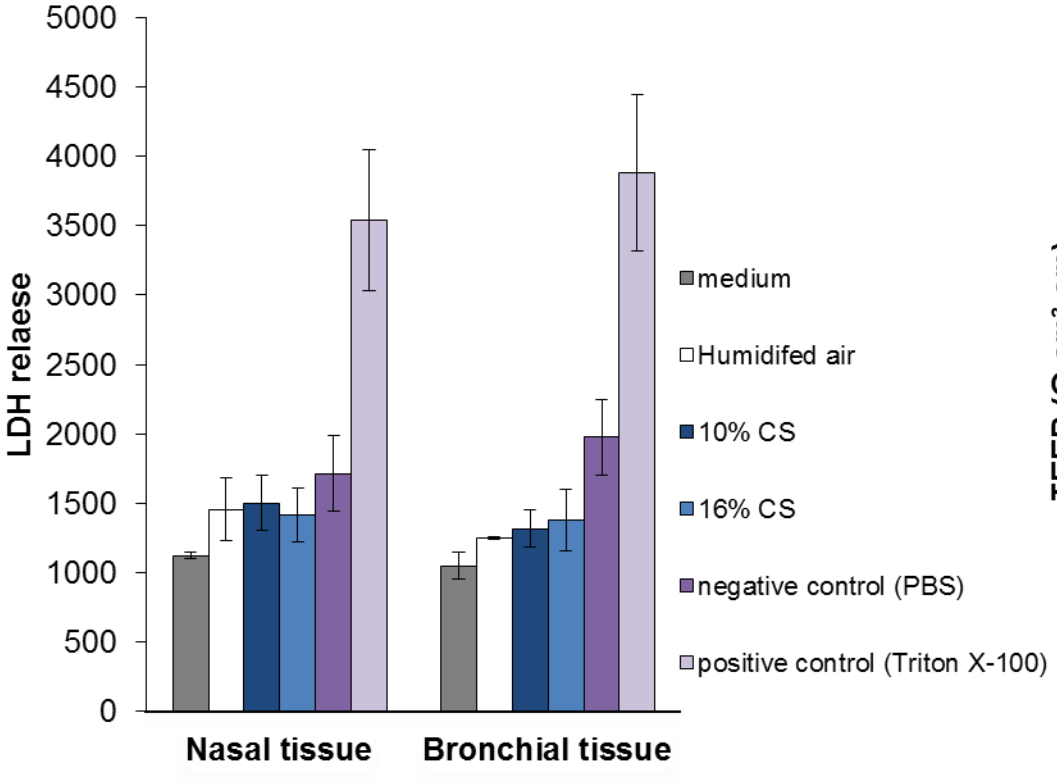
PMI RESEARCH & DEVELOPMENT

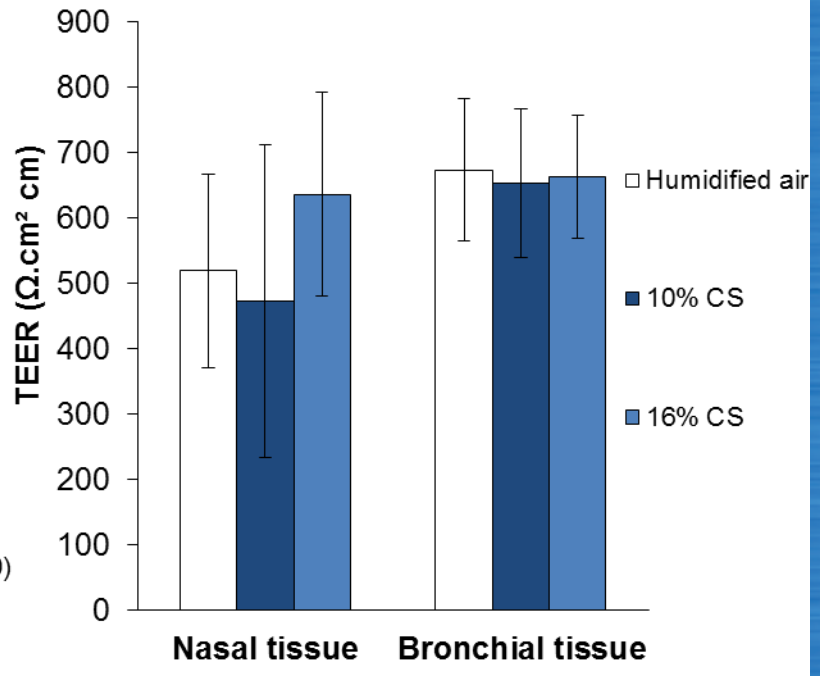
SMOKE STREET STUDY

Whole Smoke Repeated Exposure of Organotypic Cultures of Human Primary Bronchial and Nasal Epithelial Cells

Experimental Data Production

The two doses of CS applied on both nasal and bronchial tissue cultures do not induce high toxicity or impair tissue integrity allowing the capture of systems biology endpoints.





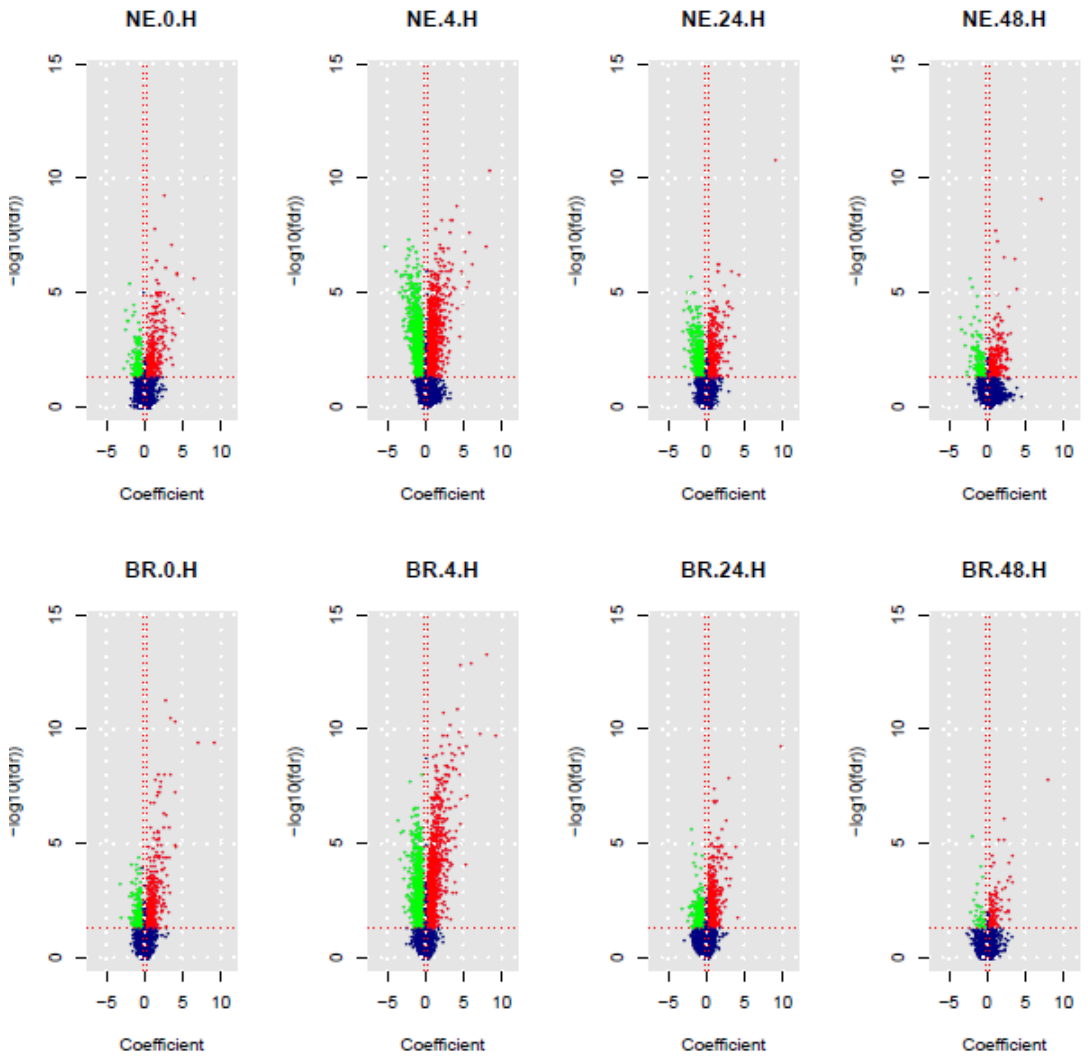
24h after exposure

PMI RESEARCH & DEVELOPMENT

SMOKE STREET STUDY

Whole Smoke Repeated Exposure of Organotypic Cultures of Human Primary Bronchial and Nasal Epithelial Cells

Volcano plot performed from the whole gene expression array demonstrating the global gene expression changes related to 16% CS which occurred during different post-exposure times (0h, 4h, 24h, 48h) in nasal and bronchial tissue cultures.



Coefficient presents log2 based fold change

Experimental Data Production

Compute Systems Response Profiles

PMI RESEARCH & DEVELOPMENT

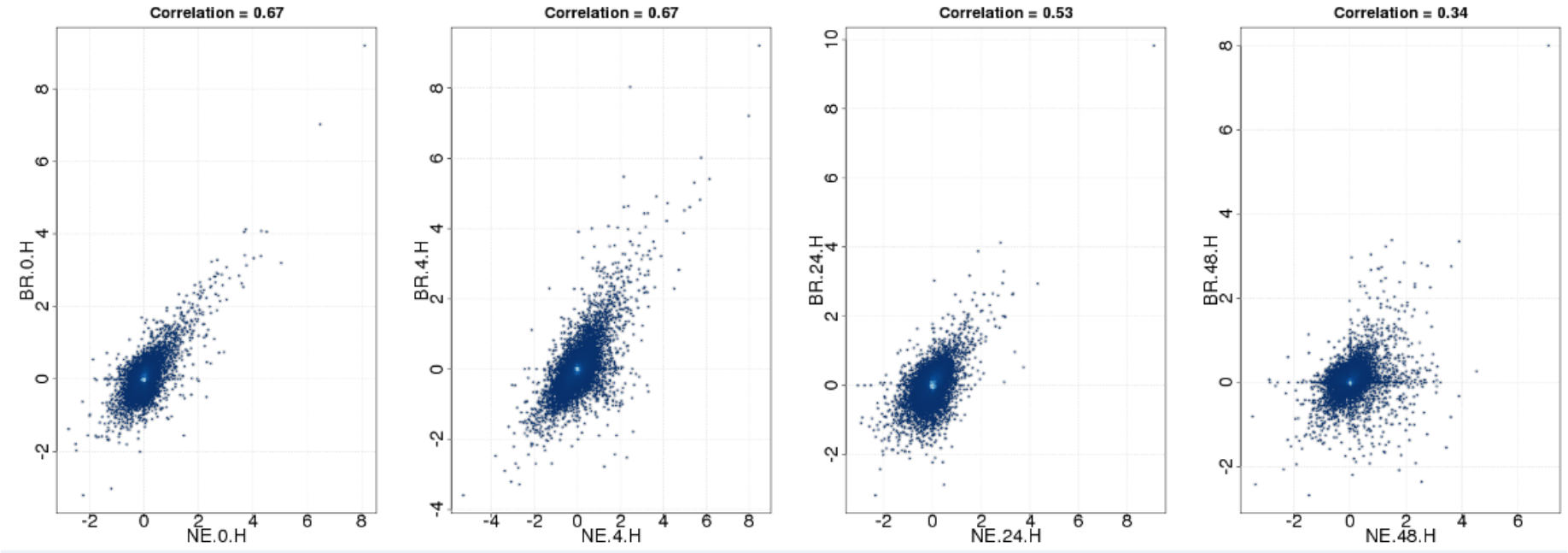
SMOKE STREET STUDY

Whole Smoke Repeated Exposure of Organotypic Cultures of Human Primary Bronchial and Nasal Epithelial Cells

Experimental Data Production → Compute Systems Response Profiles

Comparison of the gene fold-changes across the various post-exposure times between nasal and bronchial tissues.

The strong correlations for 0h and 4h are decreasing while the post-exposure time are increasing to 24h and 48h.

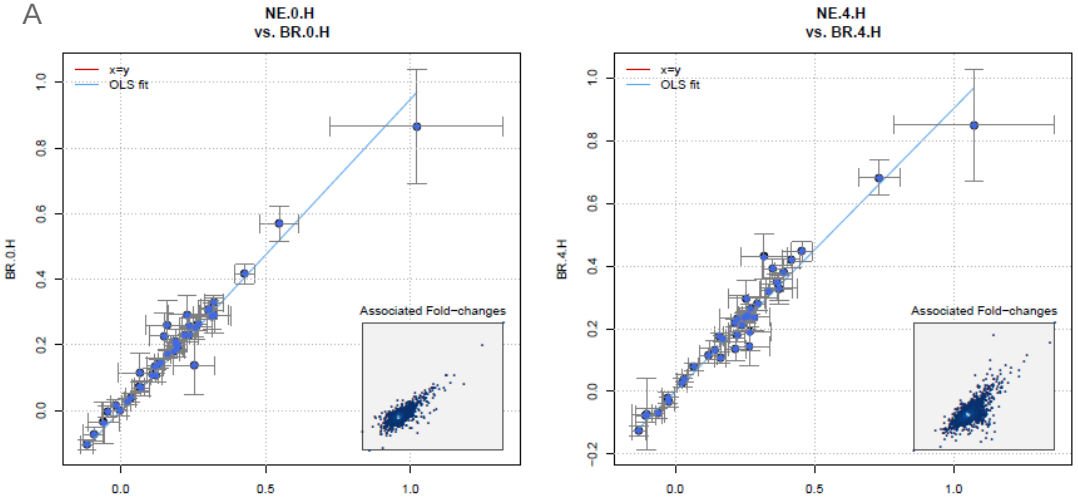


SMOKE STREET STUDY

PMI RESEARCH & DEVELOPMENT

Whole Smoke Repeated Exposure of Organotypic Cultures of Human Primary Bronchial and Nasal Epithelial Cells

A

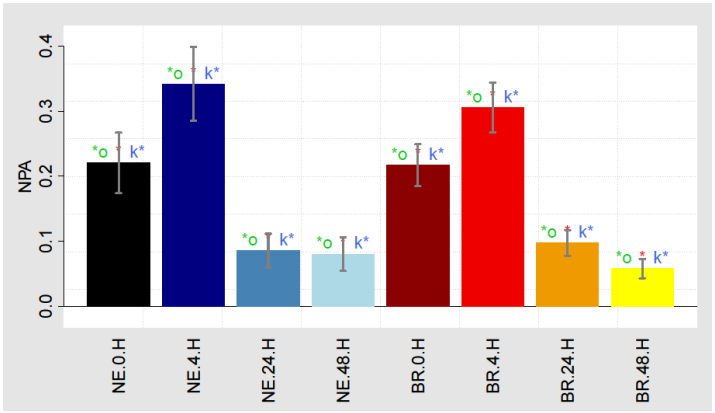


A) Strong correlation of the genes differentially regulated in the xenobiotic network between both CS-exposed nasal and bronchial tissue cultures at 0h and 4h post-exposure times.

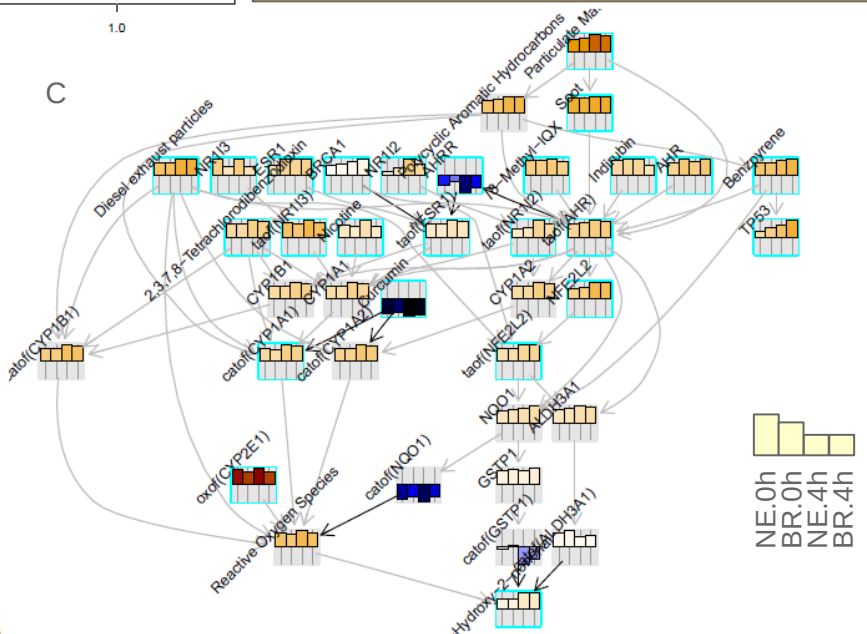
B) A similar NPA scoring of the xenobiotic network perturbations is observed at different post-exposure times in both CS-exposed nasal and bronchial tissue cultures.

C) Visualization of the xenobiotic network perturbations at 0h and 4h post-exposure times for both CS-exposed nasal and bronchial tissue cultures.

B



C



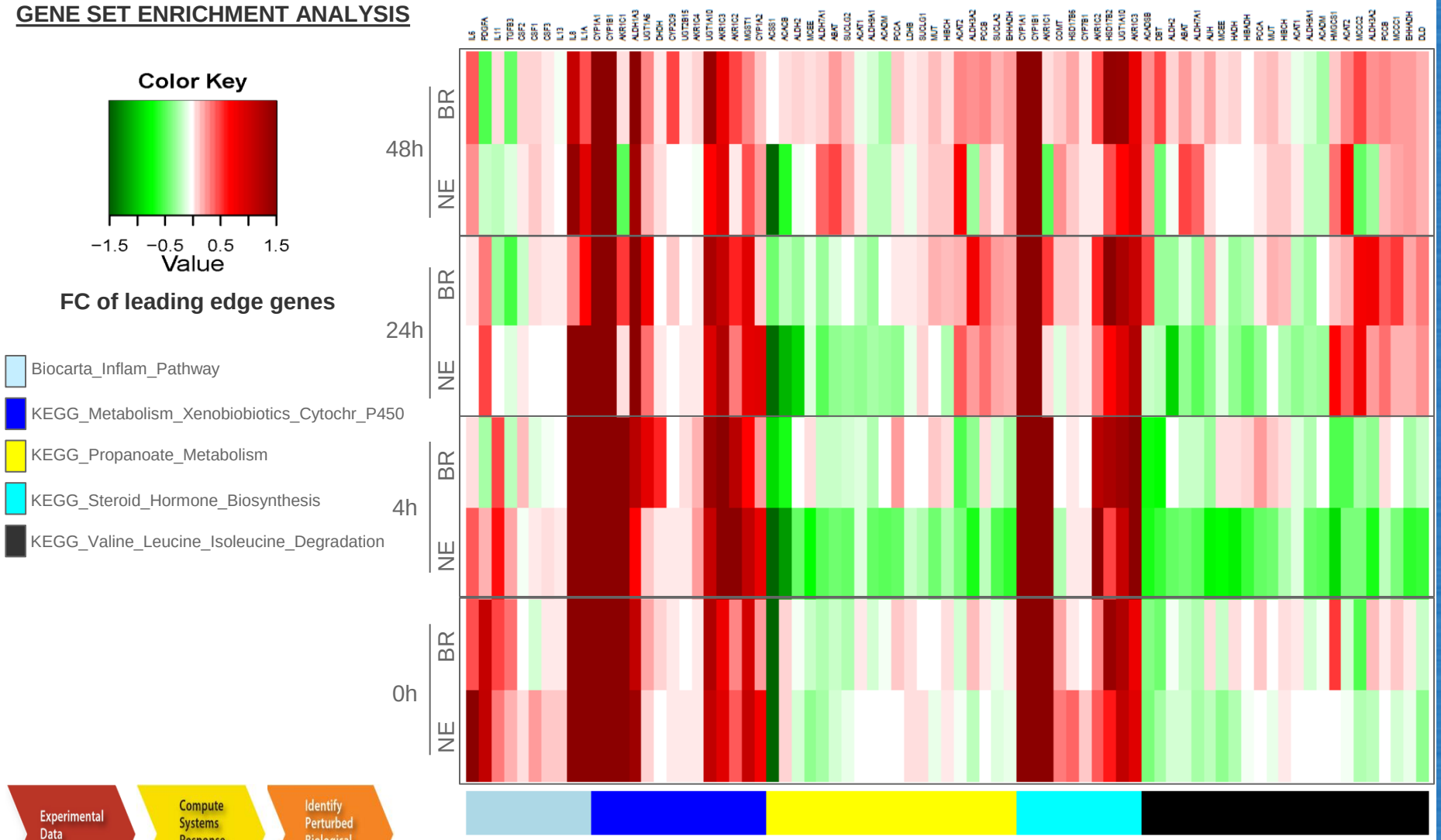
Experimental Data Production → Compute Systems Response Profiles → Identify Perturbed Biological Networks

PMI RESEARCH & DEVELOPMENT

SMOKE STREET STUDY

Whole Smoke Repeated Exposure of Organotypic Cultures of Human Primary Bronchial and Nasal Epithelial Cells

GENE SET ENRICHMENT ANALYSIS



Experimental Data Production → Compute Systems Response Profiles → Identify Perturbed Biological Networks

PMI RESEARCH & DEVELOPMENT

SMOKE STREET STUDY

Acknowledgment

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Philip Morris International R&D, Philip Morris Products S.A., Neuchâtel, Switzerland