PMI RESEARCH & DEVELOPMENT

TOPIC:

Assessing Risk Reduced Products (RRP) using Organotypic Tissues Cultures Exposed at the Air-Liquid Interface

" Systems Toxicology-Based Assessment of a RRP using Human Organotypic Tissue Cultures of Nasal and Bronchial Epithelium as well as Buccal Mucosa"

Background

2009: Publication by the U.S. National Research Council of a new strategy plan for toxicology assessment to update and advanced our knowledge on the toxicity and the MoA of environmental agents.

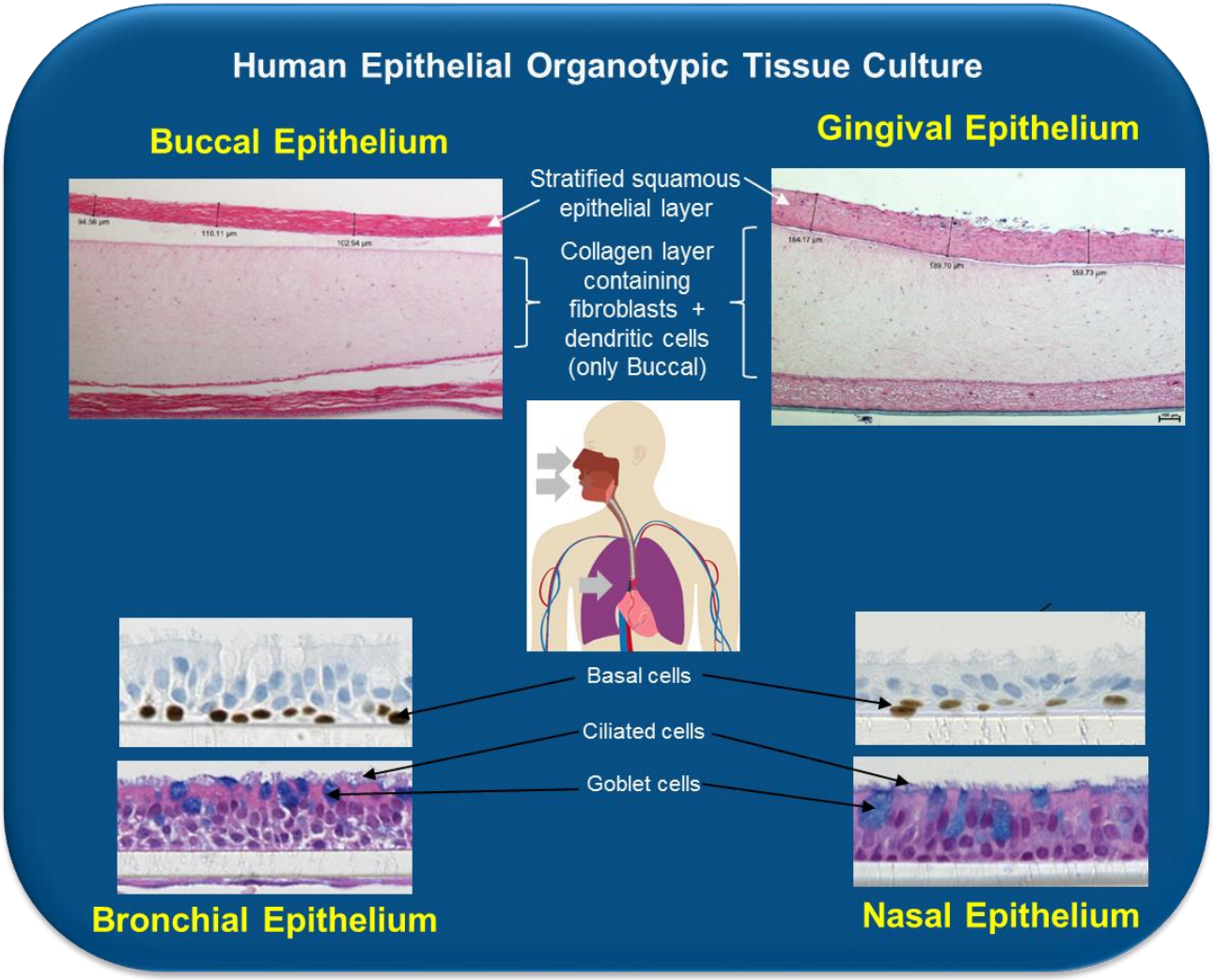
Example of recommended approaches:

- Medium and high-throughput *in vitro* screening assays
- Computational toxicology
- Systems biology
- Pharmacokinetic modeling

2009: European Commission published a report on «Alternative Testing Strategies for «Replacing, Reducing, and Refining» use of animals in research.

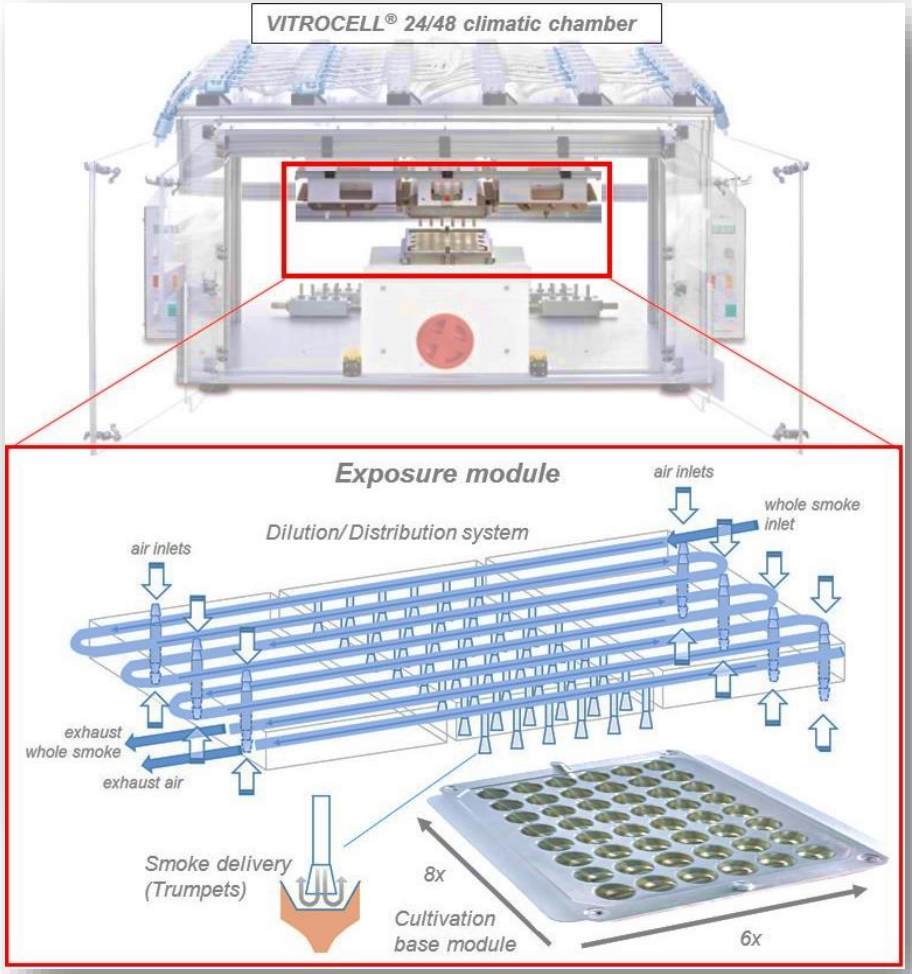
- Human organotypic tissues based on primary cells cultured in three dimensions, with proper cell-cell contact, recapitulating biological functions (e.g. mucus secretion, mucus ciliary clearance,..) and allowing whole cigarette smoke exposure at the air-liquid interface.

Human Organotypic Epithelial Tissue Cultures



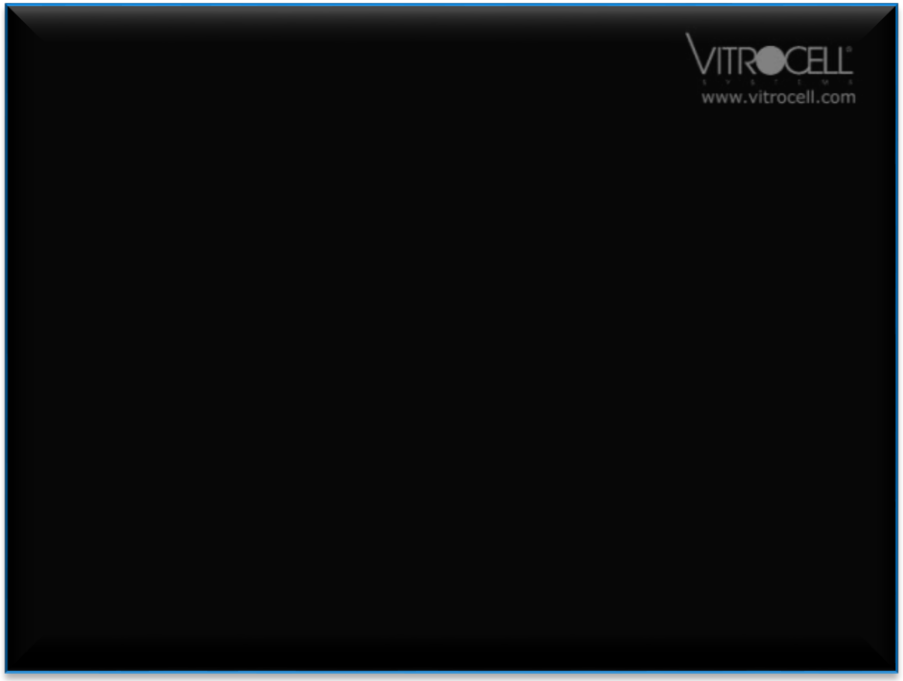
Whole Smoke Exposure System

- ❑ Humidification station
- ❑ Microbalance
- ❑ Climatic chamber
- ❑ Eight different dilutions in parallel
- ❑ Six replicates per channel



Whole cigarette smoke/aerosol exposure system (Vitrocell®)

VITROCELL® EXPOSURE SYSTEM



VITROCELL® DEPOSITION SENSOR

MOVIES

Organotypic tissue cultures: How close are they from *in vivo*?

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

Mathis C, Poussin C, Weisensee D, Gebel S, Hengstermann A, Sewer A, Belcastro V, Xiang Y, Ansari S, Wagner S, Hoeng J, Peitsch MC.
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- **Systems approaches evaluating the perturbation of xenobiotic metabolism in response to cigarette smoke exposure in nasal and bronchial tissues.**

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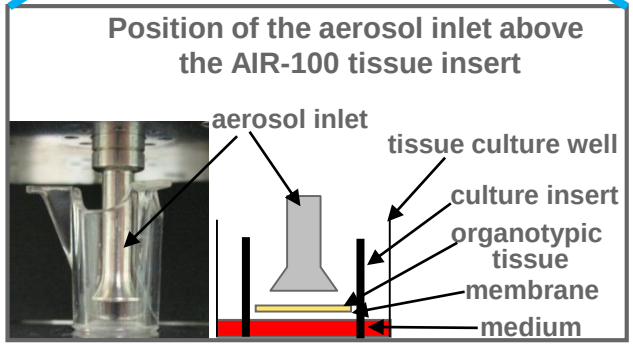
Schlage WK, Iskandar AR, Kostadinova R, Xiang Y, Sewer A, Majeed S, Kuehn D, Frentzel S, Talikka M, Geertz M, Mathis C, Ivanov N, Hoeng J, Peitsch MC.
Toxicol Mech Methods. 2014 Jul 21:1-37.

Organotypic Cultures of Human Primary Bronchial Epithelial Cells Exposed to Whole Smoke

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



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

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…

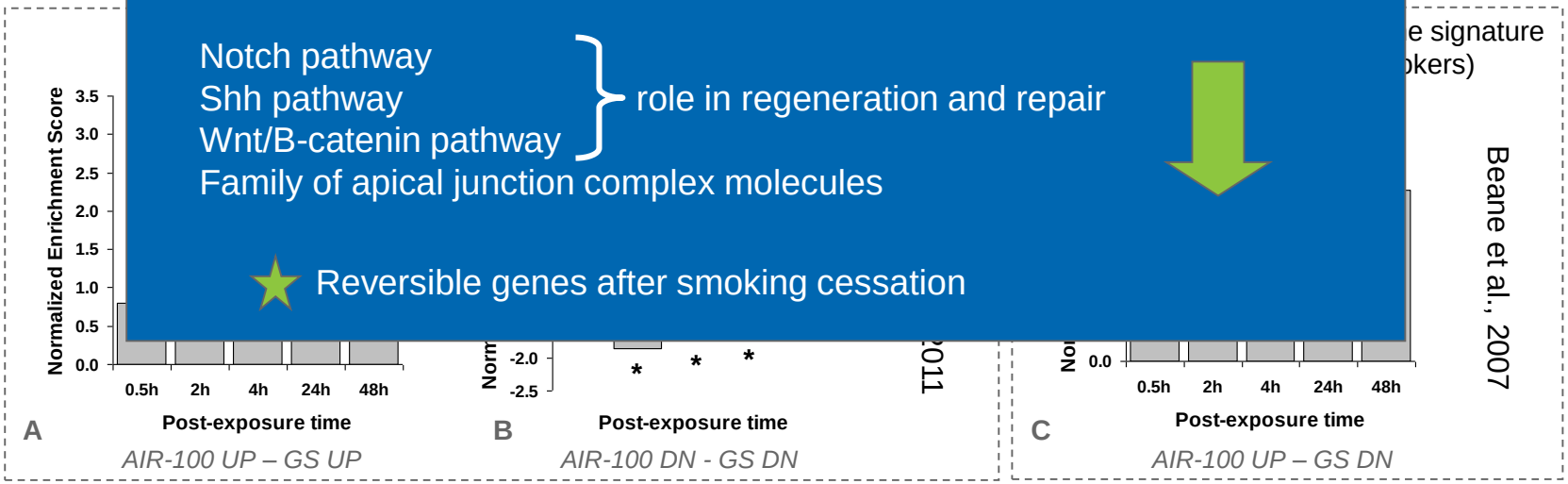
Functional Analysis

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



PMI RESEARCH & DEVELOPMENT

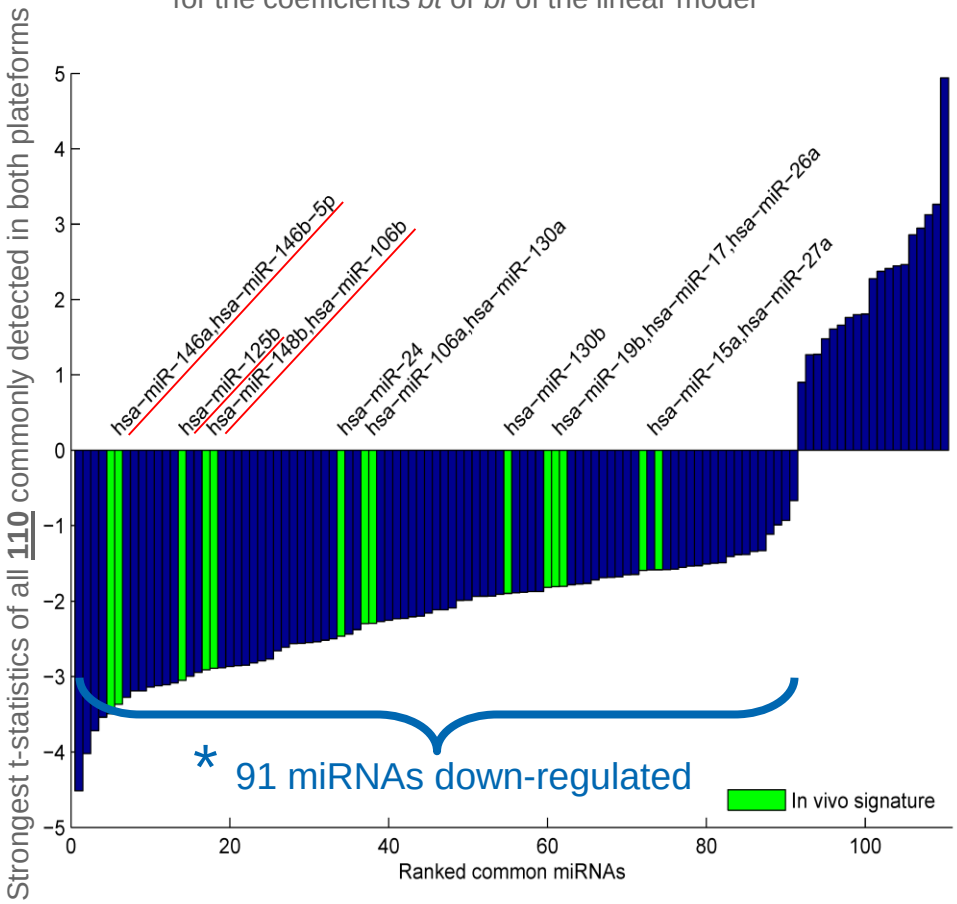
IN VITRO / IN VIVO COMPARISON – MATTEK STUDY

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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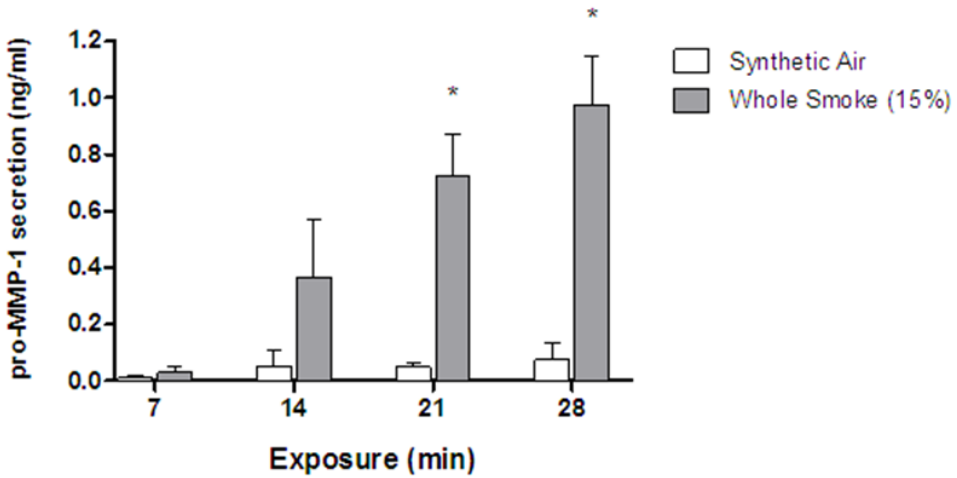
IN VITRO / IN VIVO COMPARISON – MATTEK STUDY

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.

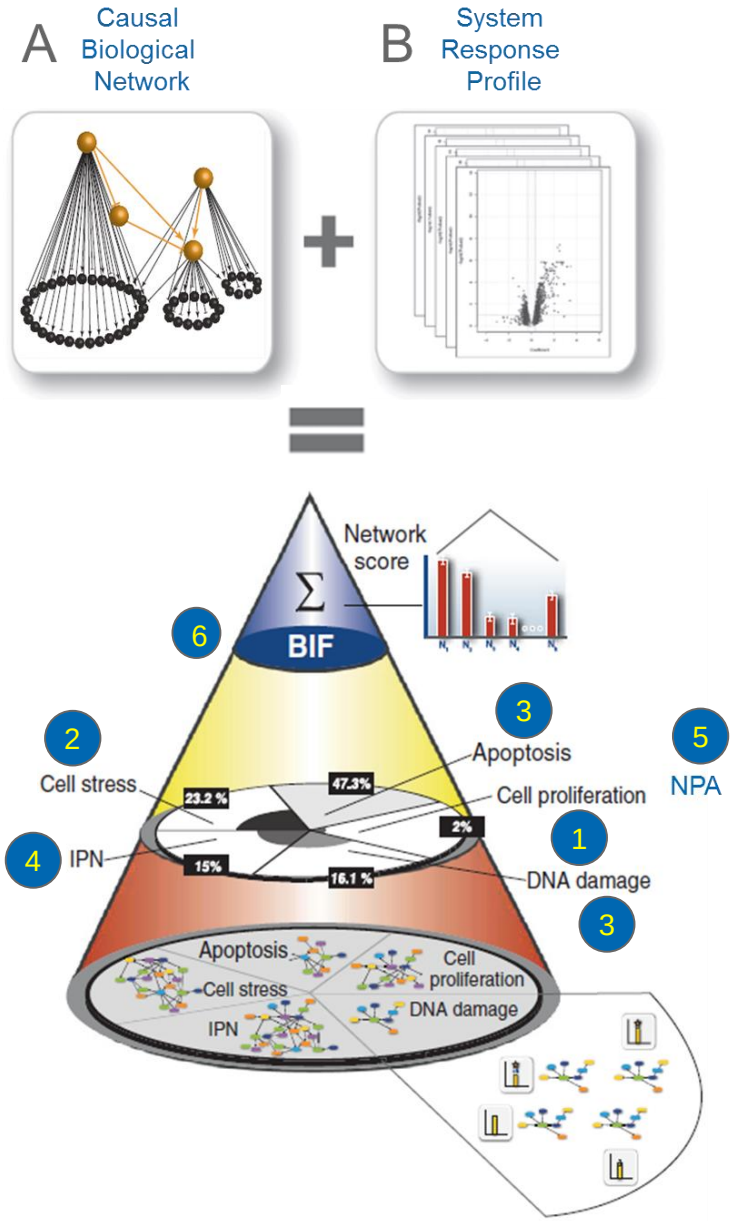
Human organotypic bronchial epithelial cells exposed to CS resemble bronchial epithelium from human smokers

- 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 Jan 25

Systems Toxicology Assessment of Whole Smoke Exposure

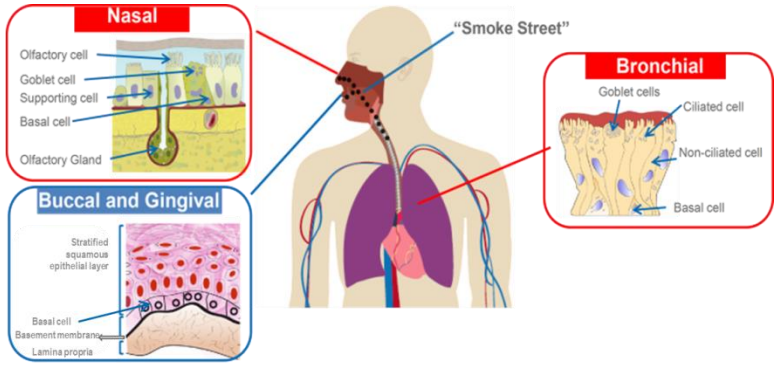


1. **Construction of a computable cell proliferation network for non-diseased lung tissue.** *J. Westra, et al.* BMC Systems Biology, 2011 Jul 5:105.
2. **Construction of a computable cellular stress network for non-diseased lung and cardiovascular tissue.** *W.K. Schlage, et al.* BMC Systems Biology, 2011 Oct 5:168.
3. **Construction of a Computable Network Model for DNA Damage, Cell Death, Autophagy, and Senescence.** *S. Gebel, et al.* Bioinformatics and Biology Insights 2013 7:97-117.
4. **A modular cell-type focused inflammatory process network model for non-diseased pulmonary tissue.** *J. Westra, et al.* Bioinformatics and Biology Insights 2013 Jun 20; 7:167-92.
5. **Assessment of network perturbation amplitude by applying high-throughput data to causal biological networks.** *F. Martin, et al.* BMC Systems Biology 2012, 6:54.
6. **Quantification of biological network perturbations for mechanistic insight and diagnostics using two-layer causal models assessment of biological impact using transcriptomic data and mechanistic network models.** *F. Martin, et al.* BMC Bioinformatics. 2014 Jul 11;15(1):238.

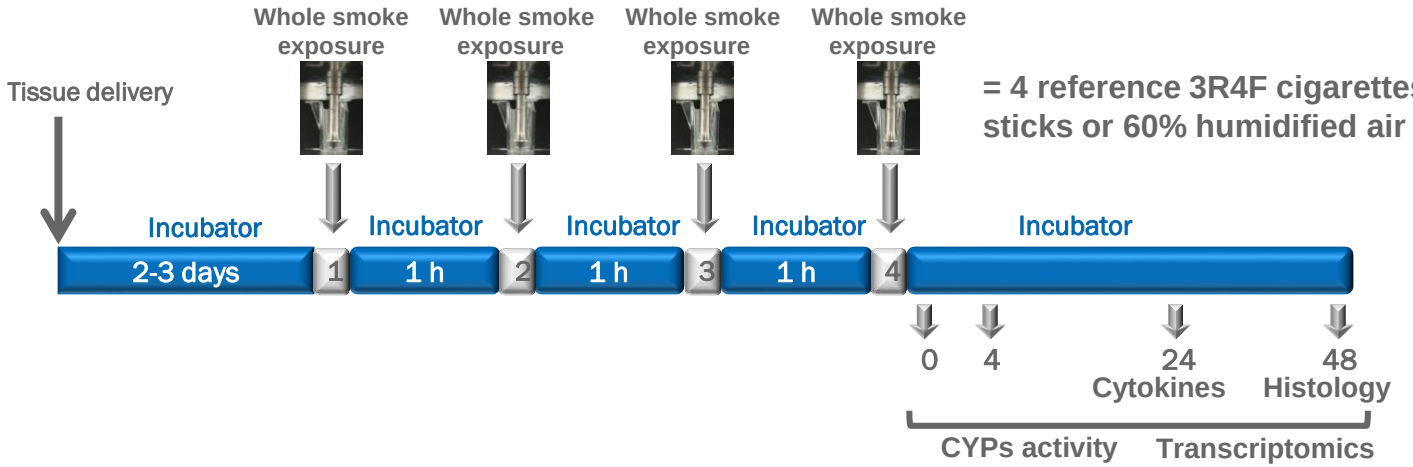
PMI RESEARCH & DEVELOPMENT

Repeated Whole Smoke Exposure of Organotypic Cultures Derived from Human Primary Epithelial Cells

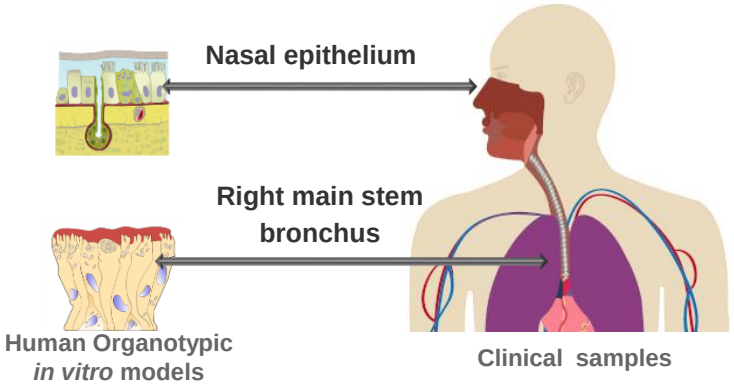
FOUR HUMAN IN VITRO MODELS



EXPERIMENTAL DESIGN



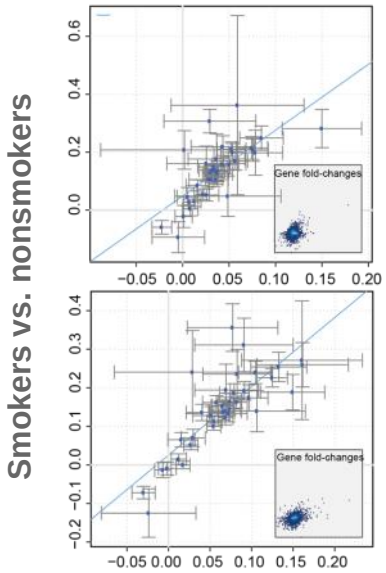
Comparison of Clinical Samples from Smokers to CS Exposed Nasal and Bronchial Organotypic Cultures



Healthy Non-smoker (n=14) Age	Healthy Smoker (n=13) Age	Smoker Pack-years
31.6 ± 10.8	35.4 ± 9.9	10.77 ± 9.3

(Zhang X. et al., Physiol. Genomics 2010)

Perturbation of the Xenobiotic metabolism network model



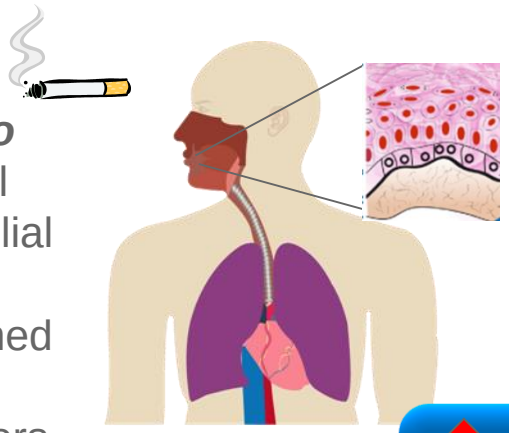
nasal

bronchial

CS vs. air exposed organotypic

Systems Approaches Evaluating the Perturbation of Xenobiotic Metabolism in Response to Cigarette Smoke Exposure in Nasal and Bronchial Tissues
Iskandar AR, Martin F, Talikka M, Schlage WK, Kostadinova R, Mathis C, Hoeng J, Peitsch MC.
Biomed Res Int. 2013 Oct 3

Comparison of Clinical Samples from Smokers to CS Exposed Buccal Organotypic Cultures

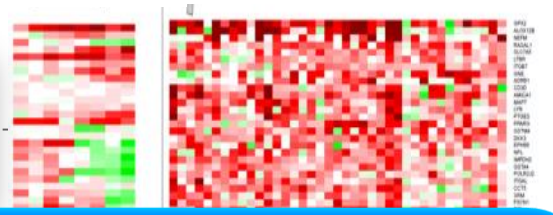


In vivo Buccal Epithelial Cells Obtained from Smokers

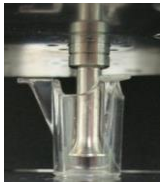
Boyle JO, et al. "Effects of cigarette smoke on the human oral mucosal transcriptome." ***Cancer Prev Res (Phila)*** **2010 Mar;3(3):266-78.** PMID: 20179299

- 40 never smokers (<100 cigarettes per lifetime)
- 40 active smokers (≥ 15 pack year exposure)
- **Buccal biopsies**
- Age- and gender-matched

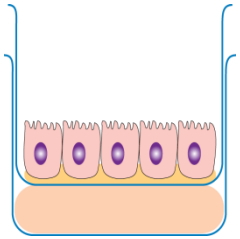
In Vitro **In Vivo**

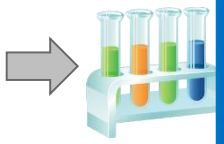


- *Metabolism of xenobiotics by P450s*
- *Steroid hormone biosynthesis*
- *Glutathione metabolism*
- *Drug metabolism*



In vitro Organotypic Buccal Epithelial Exposed to Smoke





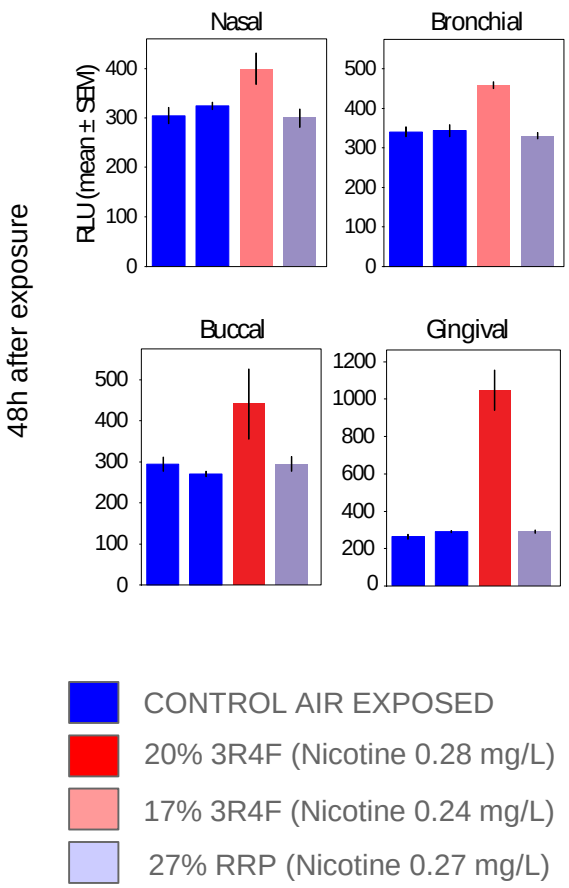
EXTRACT D
RNA, AND PRO

In vitro systems toxicology approach to investigate the effects of repeated cigarette smoke exposure on human buccal and gingival organotypic epithelial tissue cultures

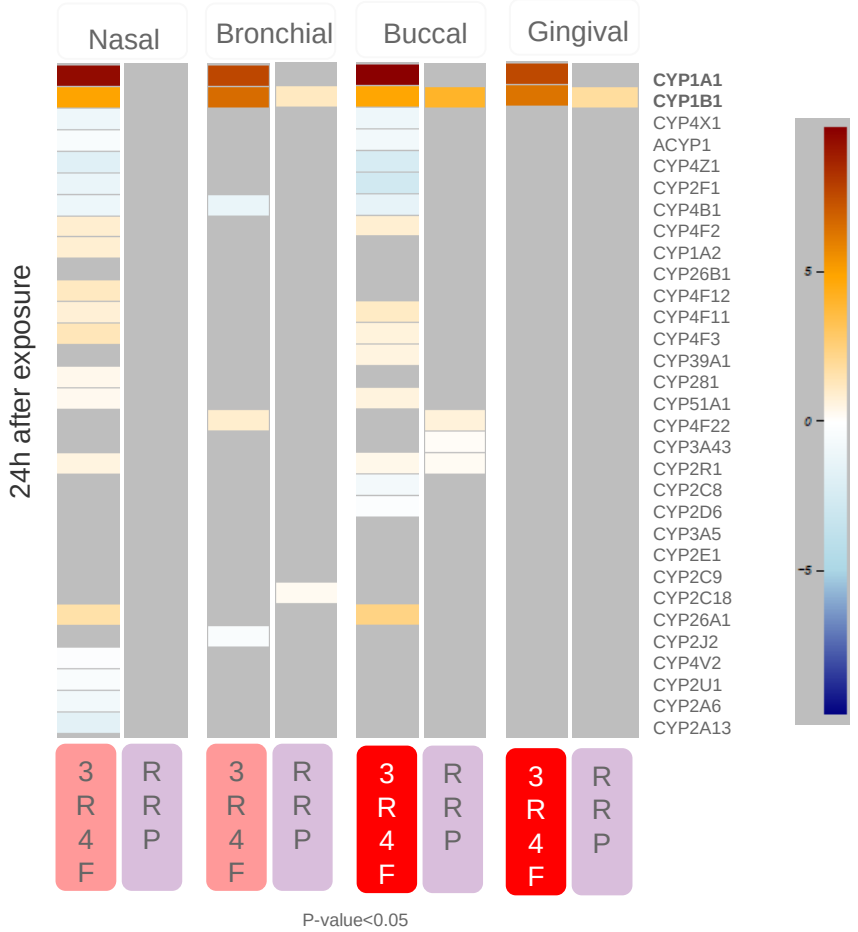
Schlage WK, Iskandar AR, Kostadinova R, Xiang Y, Sewer A, Majeed S, Kuehn D, Frentzel S,Talikka M, Geertz M, Mathis C, Ivanov N, Hoeng J, Peitsch MC. Toxicol Mech Methods. 2014 Jul 21:1-37

Repeated exposure to RRP's aerosol does not induce CYP1A1/1B1 activity and/or CYPs genes expression change

CYP1A1/CYP1B1 enzymatic activity



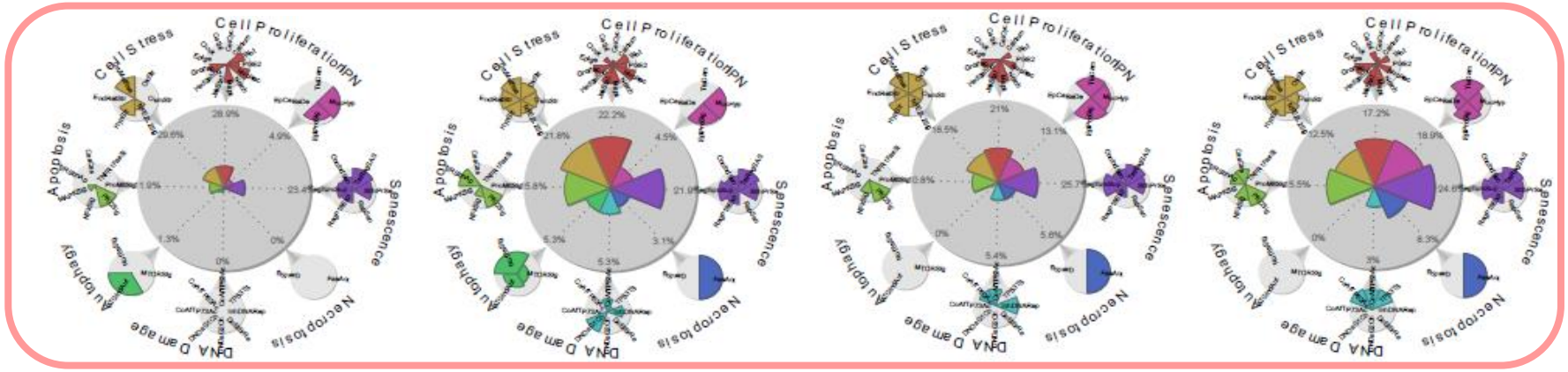
CYP genes expression



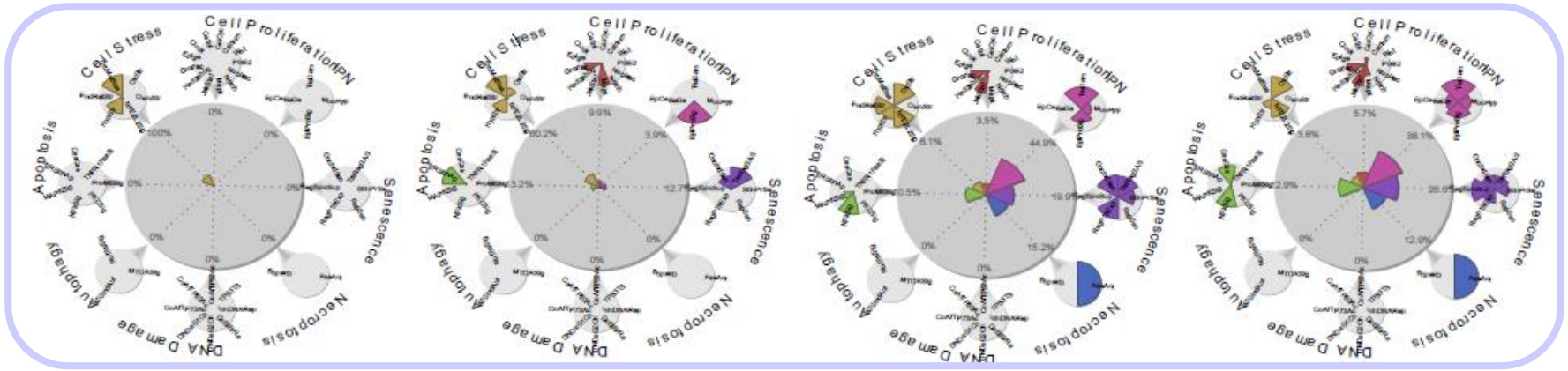
Repeated exposure to RRP's aerosol has a lower impact on network perturbations compared to conventional CS

BRONCHIAL

17% 3R4F (Nicotine 0.24 mg/mL)

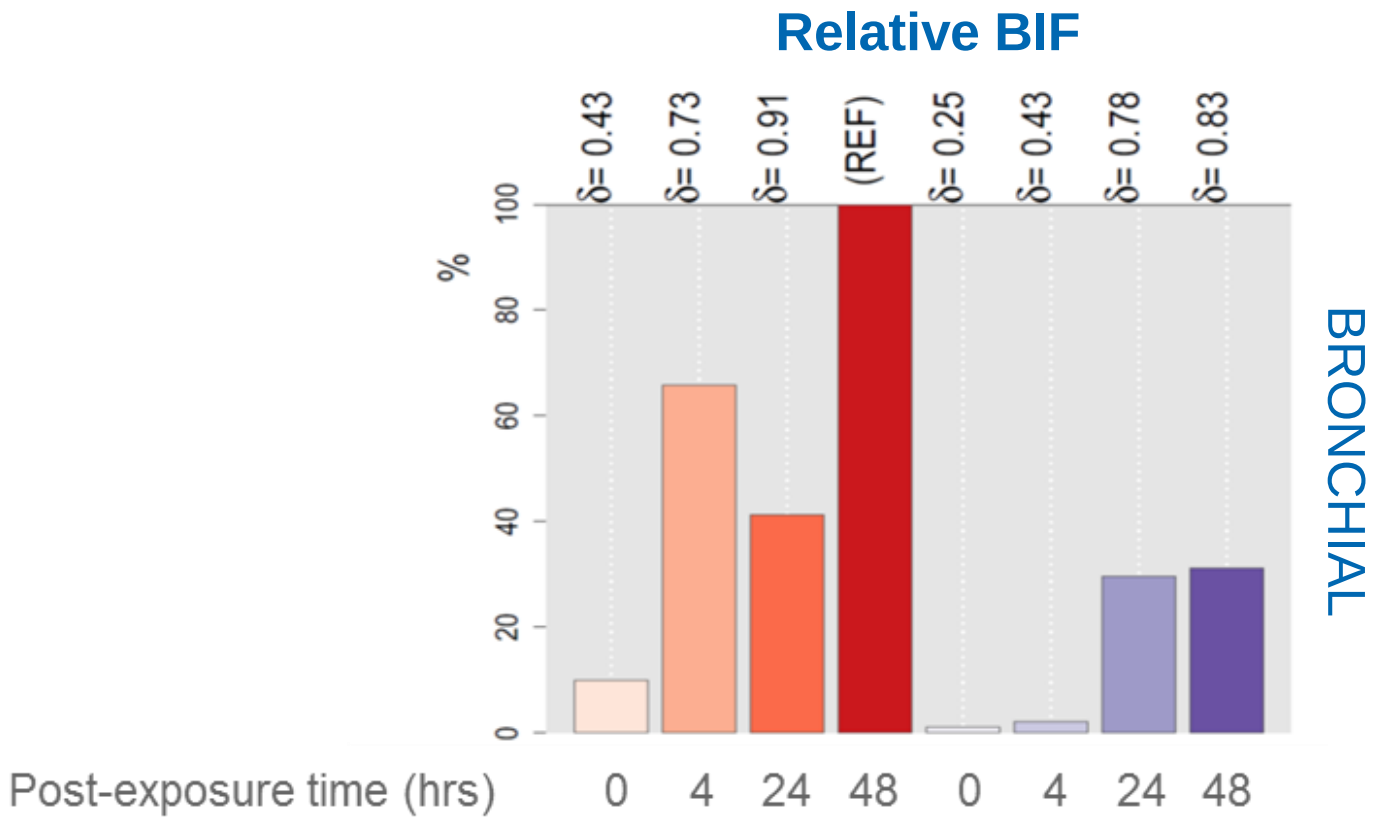


0h 4h 24h 48h

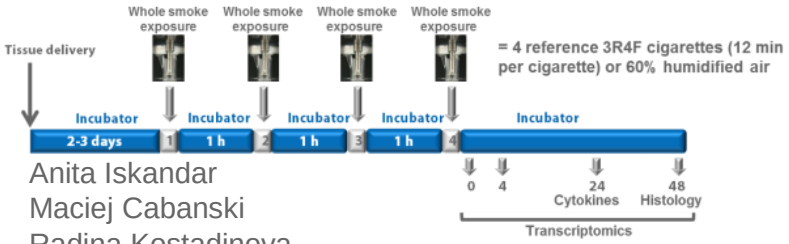


27% RRP (Nicotine 0.27 mg/mL)

At Similar Dose of Nicotine, the Biological Impact Factor of RRP Exposure Is Lower Than Conventional Cigarette



Acknowledgment



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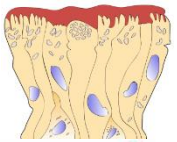

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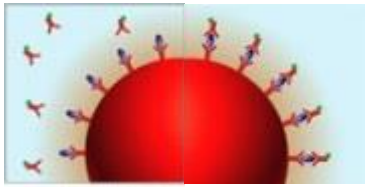

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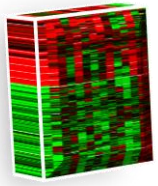

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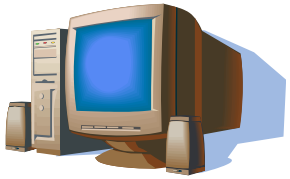

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