PMI RESEARCH & DEVELOPMENT

Identification and translation of oxidative stress biomarkers: from pre-clinical to clinical studies

Nikolai V. Ivanov, Ph.D.

Manager, Research Technologies

25 February 2016

11th Annual Biomarkers Congress

Manchester Central Convention Complex, Manchester, UK

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Talk outline

- Introduction
- A case study of biomarkers of oxidative stress in a pre-clinical study.
- A case study of biomarkers of oxidative stress in a clinical study.
- Systems Toxicology approach to translate oxidative stress biomarkers from pre-clinical to clinical studies

Background to PMI R&D

- Smoking causes serious diseases such as cardiovascular diseases, lung cancer and chronic obstructive pulmonary disease.
- Philip Morris International is therefore developing novel products that may have the potential to reduce smoking-related disease risk compared to conventional combustible cigarettes.
- To determine whether such potentially reduced-risk products (RRP*) have the potential to reduce disease risk, we compare their biological impact with that of a combustible reference cigarette (3R4F) on a mechanism-by-mechanism basis (using Systems Toxicology approach).

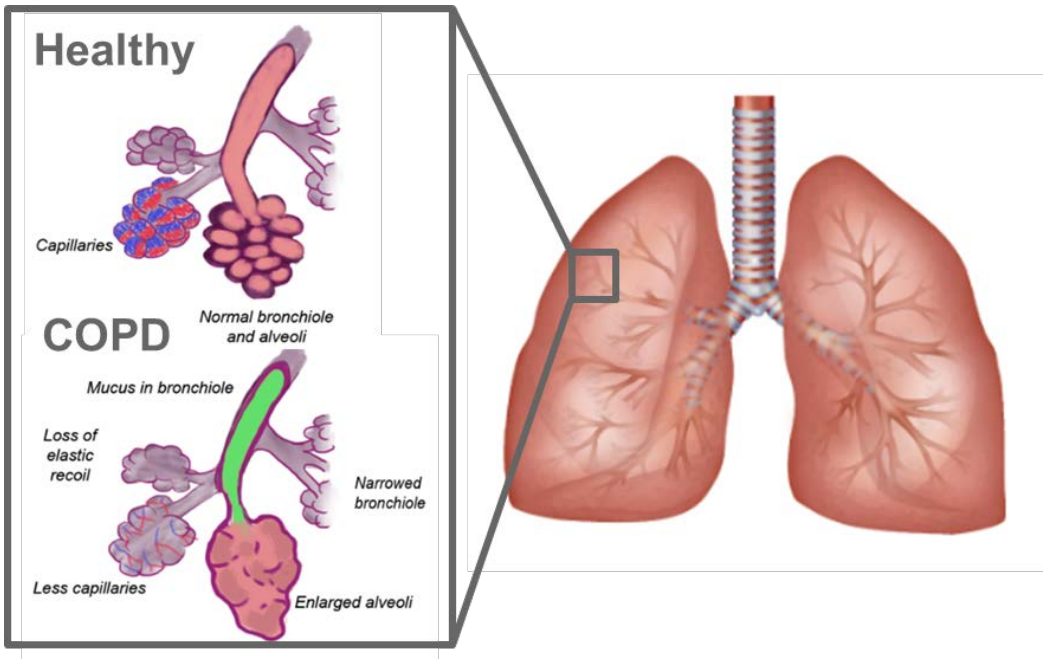
* Reduced Risk Products ("RRPs") is the term we use to refer to products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes. PMI's RRPs are in various stages of development, and we are conducting extensive and rigorous scientific studies to determine whether we can support claims for such products of reduced exposure to harmful and potentially harmful constituents in smoke, and ultimately claims of reduced disease risk, when compared to smoking cigarettes. Before making any such claims, we will rigorously evaluate the full set of data from the relevant scientific studies to determine whether they substantiate reduced exposure or risk. Any such claims may also be subject to government review and approval, as is the case in the USA today.

Terminology

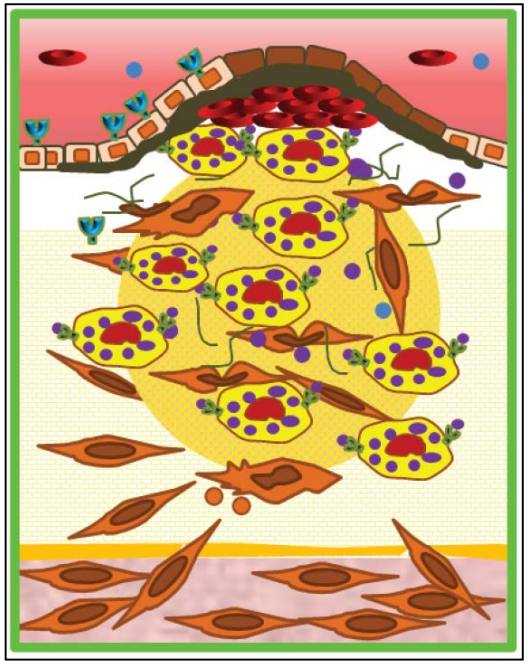
- Platform 1 Risk Reduced Product (P1 RRP)
  - Versions: THS2.2, THS2.4
- Platform 1 Modified Risk Tobacco Product (P1 MRTP) – US FDA term
- 3R4F - Reference cigarette (Univ. of Kentucky)

Smoking Induced Respiratory and Cardiovascular diseases

Respiratory disease



Cardiovascular disease



- Damage to the lung tissue, with a loss of normal elasticity of the air sacs (emphysema)
- Obstruction of small airways (bronchioles)
- Chronic bronchitis, which is marked by a thickening of the walls of the bronchi with increased mucus production

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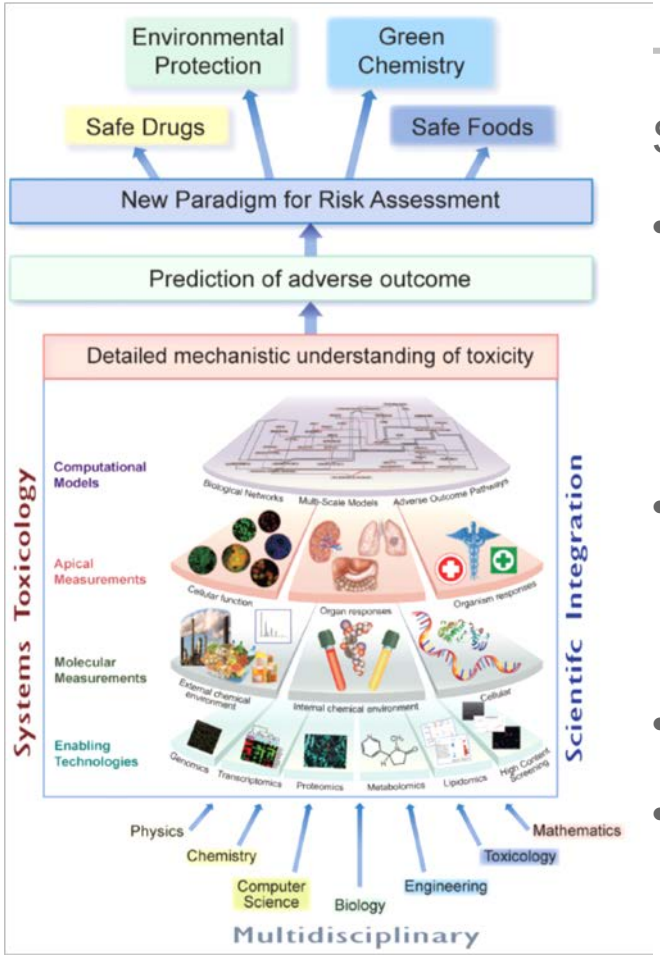
What Is Systems Toxicology?



Systems Toxicology is:

- the integration of the classic toxicology paradigm with the quantitative analysis of many molecular and functional changes occurring across multiple levels of biological organization.
- aimed at developing a detailed mechanistic as well as dynamic understanding of toxicological processes (*Research*).
- enabling inter-species and system translation.
- part of a new paradigm for risk assessment (*Product Assessment*).

Sturla SJ, Boobis AR, FitzGerald RE, Hoeng J, Kavlock RJ, Schirmer K, Whelan M, Wilks MF, Peitsch MC (2014) Systems toxicology: from basic research to risk assessment. *Chemical research in toxicology 27: 314-329*

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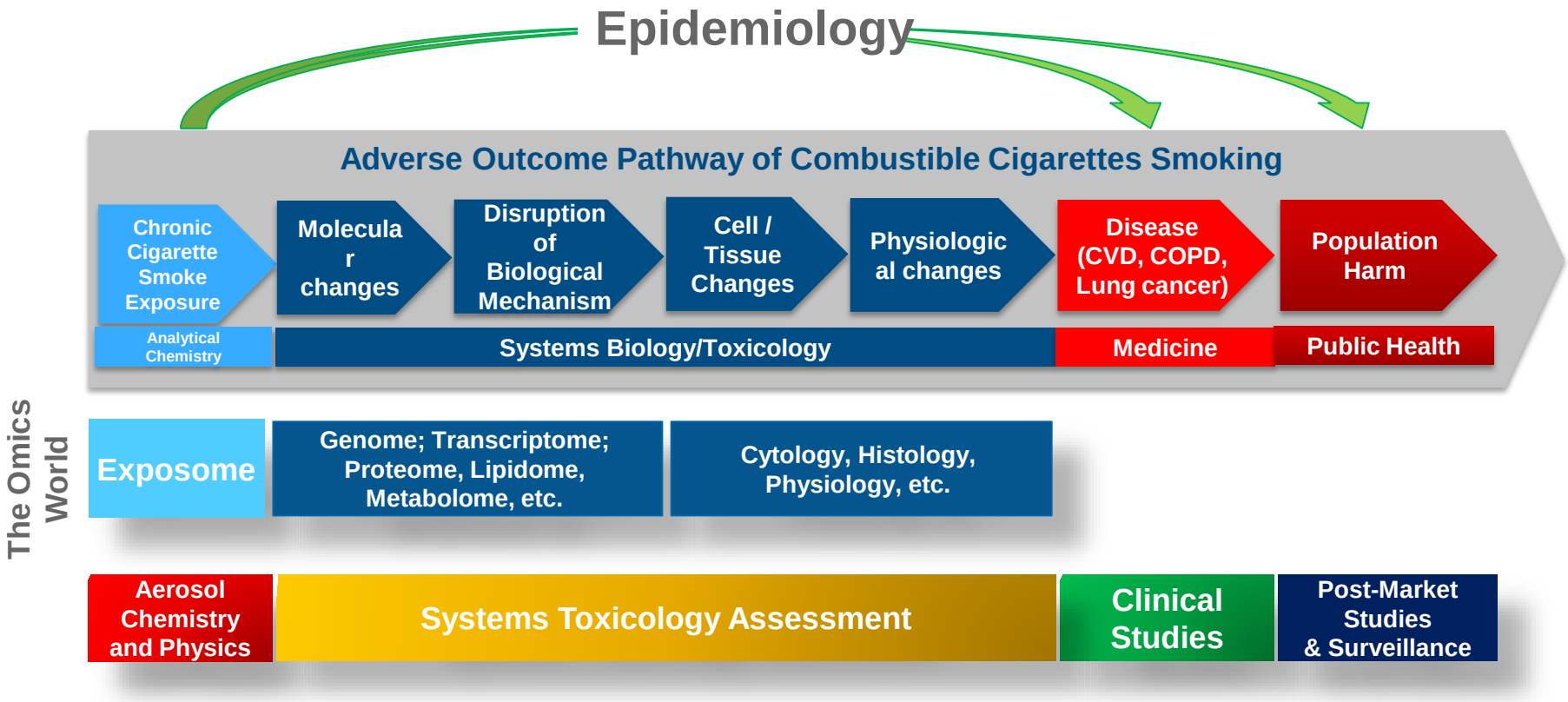
From Exposure to Population Harm: A Causal Chain of Events

High Level Adverse Outcome Pathway (AOP) of Cigarette Smoke Exposure



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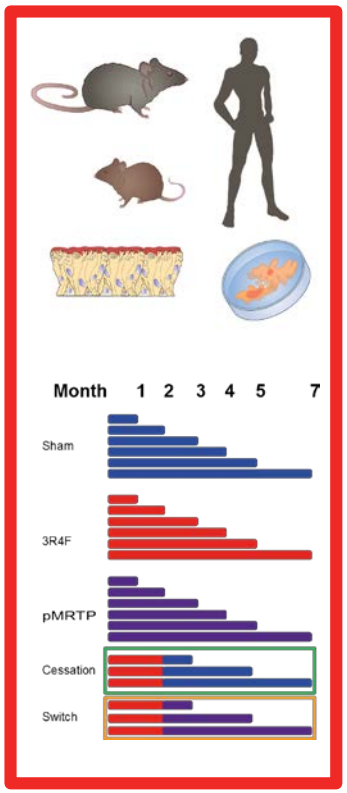
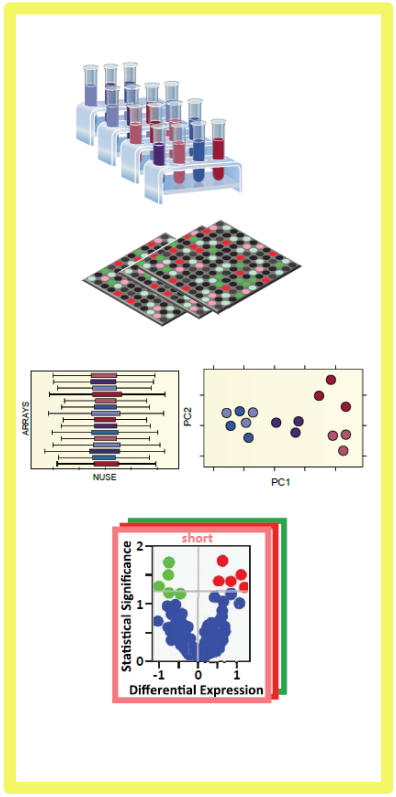
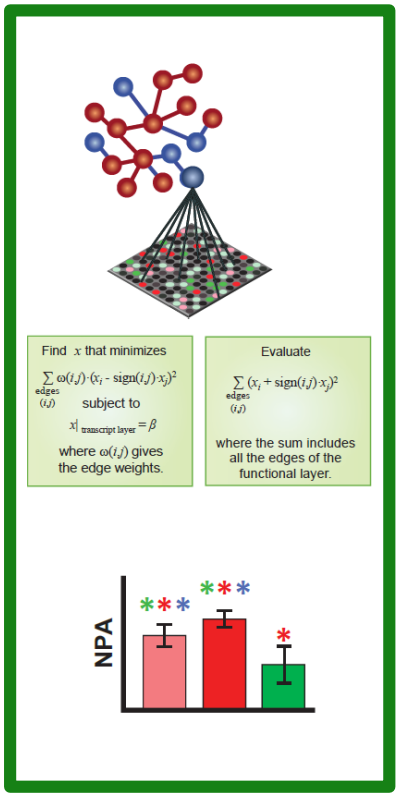
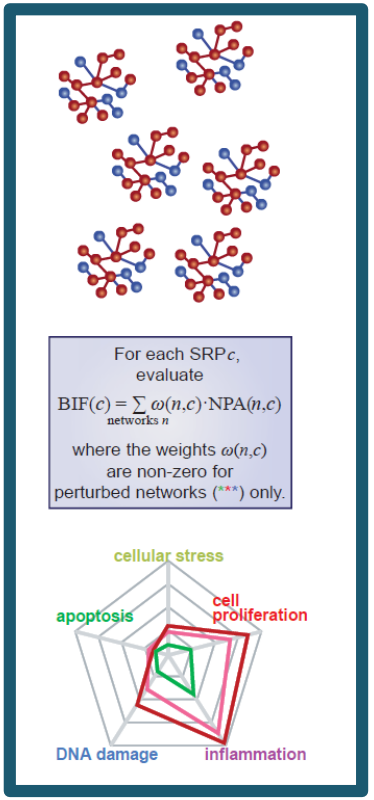
Quantitative Mechanism-Based Systems Impact Assessment

Experimental data production → **Compute systems response profiles** → **Identify perturbed biological networks** → **Compute network perturbation amplitudes** → **Compute product biological impact**



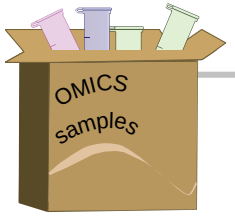


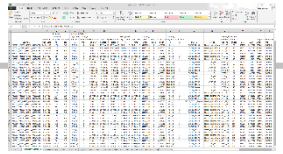




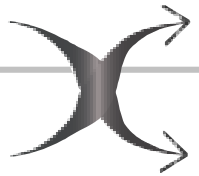
Hoeng J, Deehan R, Pratt D, Martin F, Sewer A, Thomson TM, Drubin DA, Waters CA, de Graaf D, Peitsch MC (2012) A network-based approach to quantifying the impact of biologically active substances. *Drug discovery today 17: 413-418*

"Omics" Workflows





Biobanking and metadata preparation



Randomization

Retrieve samples in randomization order

Proteomics Workflow LC-MS (iTRAQ)

Lysis and protein precipitation

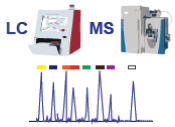
Protein concentration determination and QC

Normalization

Equal protein amounts for all samples

8-plex | reduction blocking | digestion labelling

Peptide pool



Comparison of iTRAQ ratios (exp vs. sham) and statistical analysis

Lipidomics Workflow

Folch extraction | Fong extraction | Deems extraction

+ spike-in (non-endogeneous synthetic internal standards)

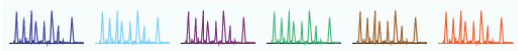
Shotgun Lipidomics (direct infusion)

Targeted Lipidomics (LC-MS/MS)

MPIS-based methods (+/- ion mode)

NL-based methods (+ ion mode)

LC-MRM (- ion mode | + ion mode | - ion mode | - ion mode)



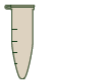
CE, PC, LPC, PS, PE, PG, PI, PA, SM, DAG | TAG | SPH | CER | GL | EC

Export raw data (masses and counts of identified molecules)

Calibration line to determine dynamic range

Quantify endogenous lipids using internal standards

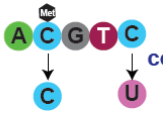
Genomics Workflow



DNA extraction

Fragmentation

Barcoded adapter ligation



Bisulfite conversion

Library amplification

PhiX DNA spike-in to determine the sequencing error rate

Sequencing

Mapping to converted references

Transcriptomics Workflow

Randomization

RNA extraction

Randomization

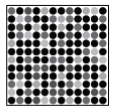
QC RNA quality and quantity

Microarray preparation

QC amplification process

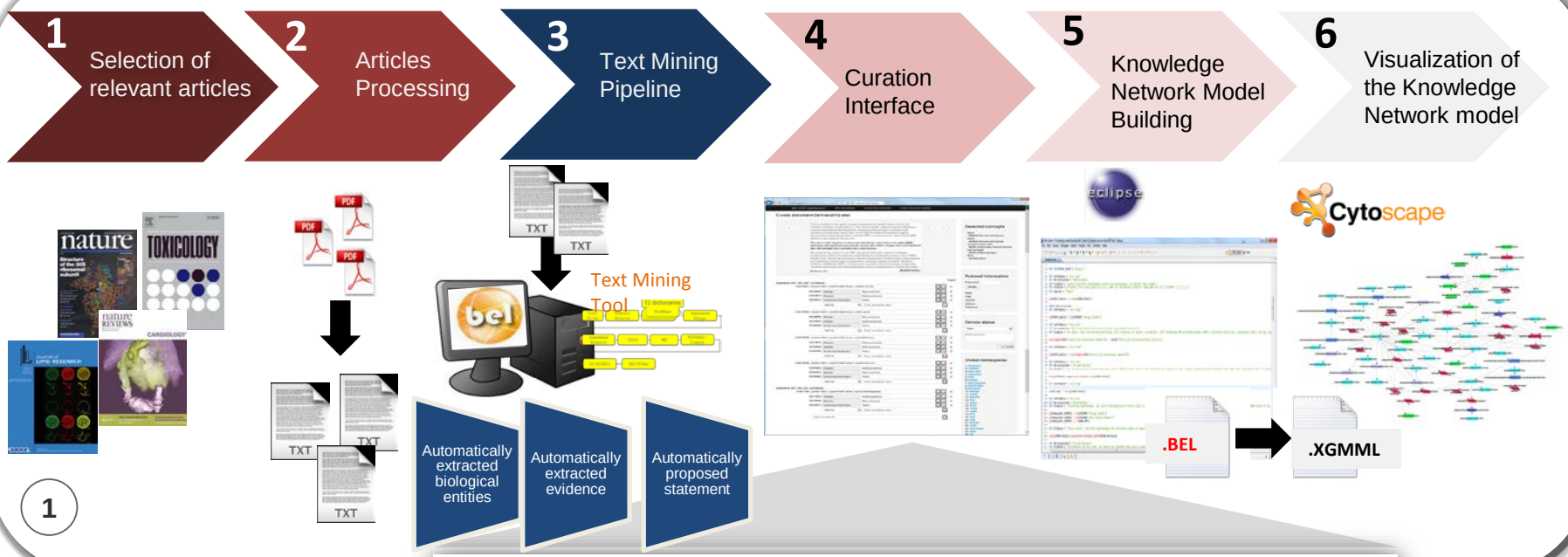
Hybridization

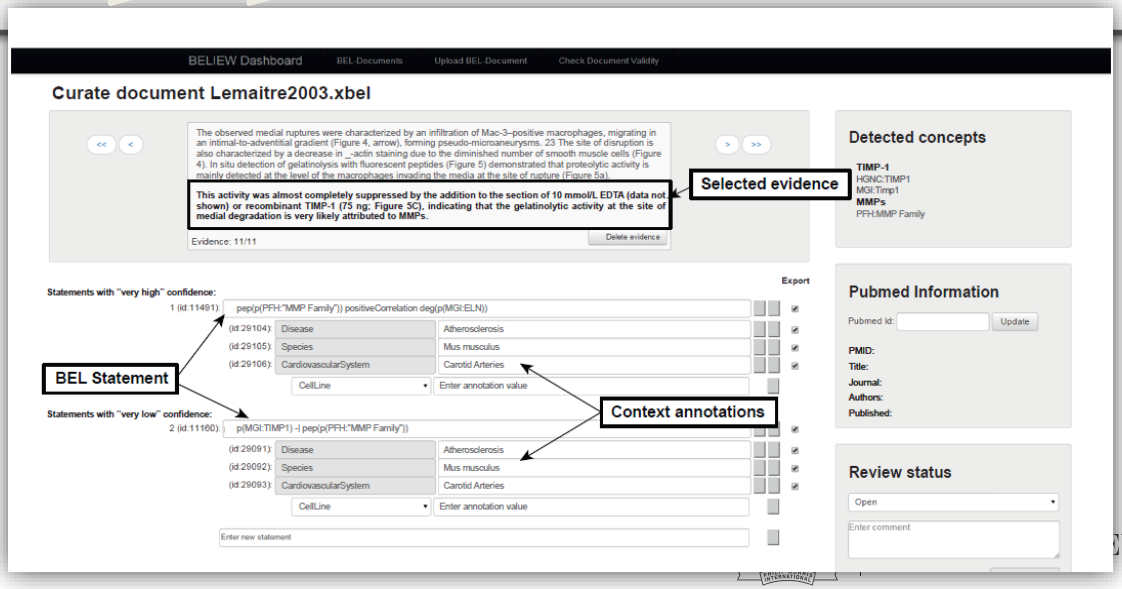
Scanning



visual QC microarray artifacts

Biological Network Model-Development Process

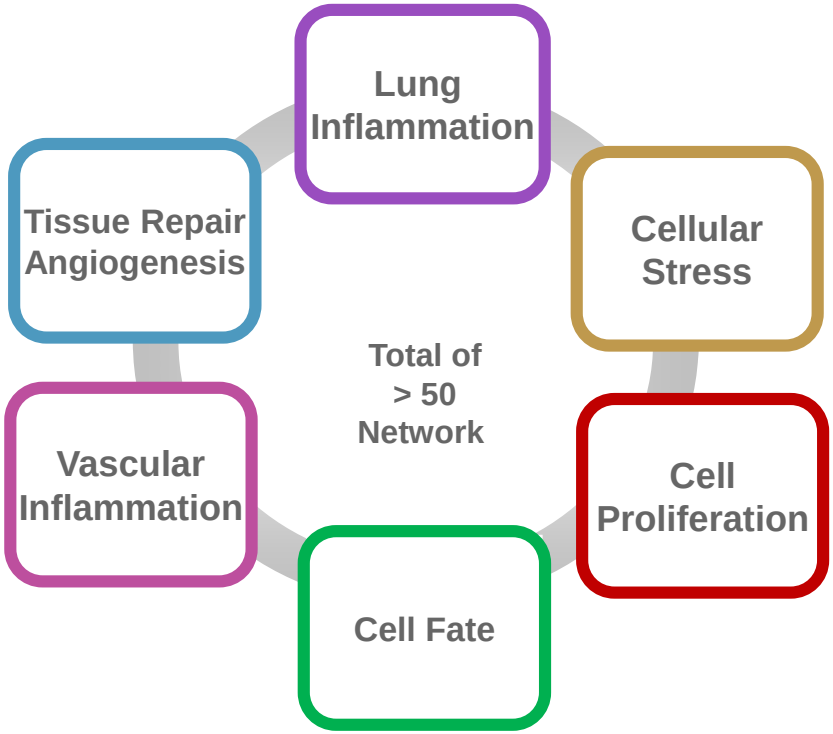




Szostak J, Ansari S, Madan S, Fluck J, Talikka M, Iskandar A, De Leon H, Hofmann-Apitius M, Peitsch MC, Hoeng J (2015) Construction of biological networks from unstructured information based on a semi-automated curation workflow. ***Database 2015: bav057***

Build and Maintain Biological Network Models

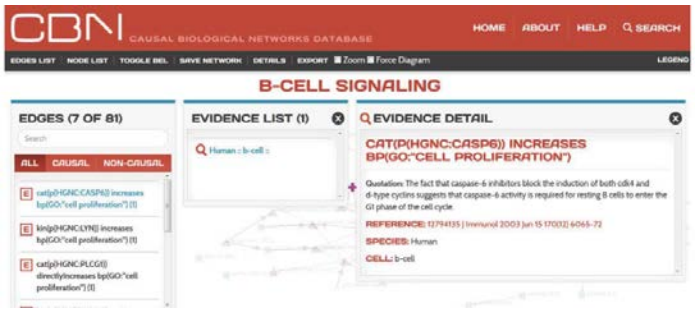
6 publications available upon request



- Identify mechanisms affected by the exposure.
- Build computable Network Models.
- Maintain Network Models over time as know new knowledge accumulates.

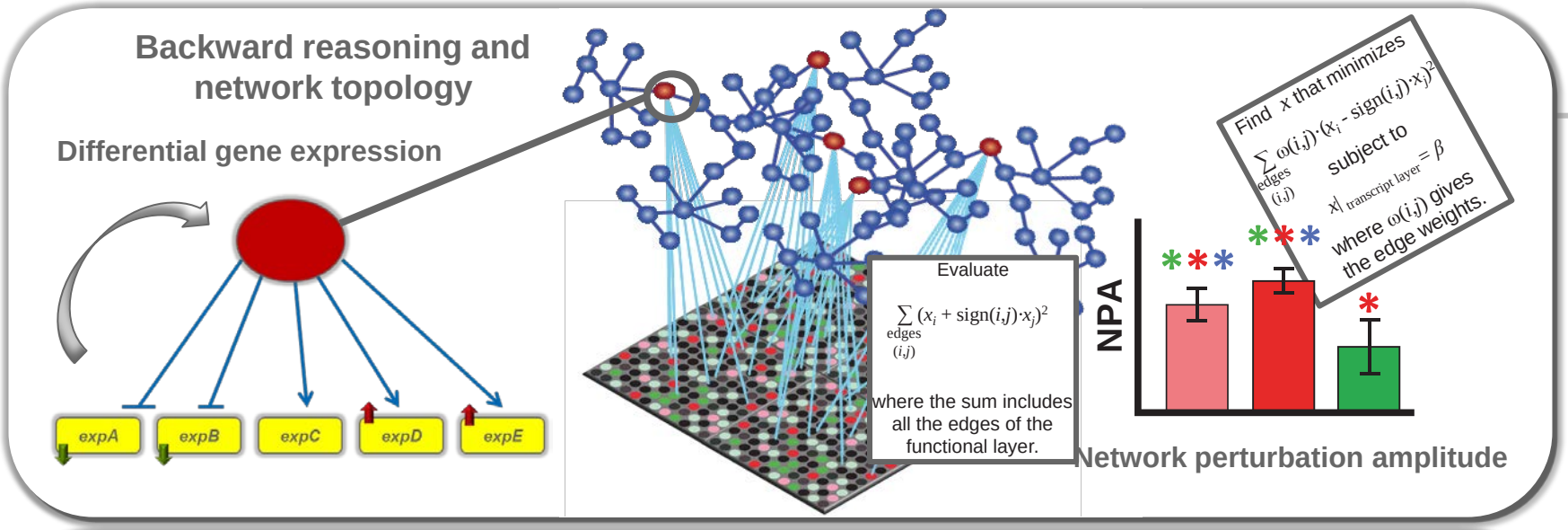
The network models are available at:

- http://www.causalbionet.com/

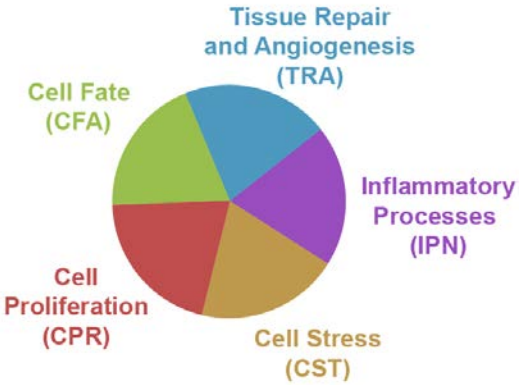


Boué S, Talikka M, Westra JW, Hayes W, Di Fabio A, Park J, Schlage WK, Sewer A, Fields B, Ansari S (2015) Causal biological network database: a comprehensive platform of causal biological network models focused on the pulmonary and vascular systems. ***Database 2015: bav030***

Network Scoring to Quantify Biological Impact



Network families



Calcium
Cell Cycle
Cell Interaction
Clock
Epigenetics
Grwoth Factor
Hedgehog
Hox
Jak Stat
Mapk
MTor
Notch
Nuclear Receptors
PGE2
Wnt

Apoptosis
Autophagy
Necroptosis
Response to DNA Damage
Senescence

Endoplasmic Reticulum Stress
Hypoxic Stress
NFE2L2 Signaling
Osmotic Stress
Oxidative Stress
Xenobiotic Metabolism Response

Angiogenesis
ECM Degradation
Endothelial Innate Immune Activation
Fibrosis
Immune Regulation Of Tissue Repair
Wound Healing

B-cell Signaling
Cytotoxic T-cell Signaling
Dendritic Cell Signaling
Epithelial Innate Immune Activation
Epithelial Mucus Hypersecretion
Macrophage Signaling
Mast Cell Activation
Megakaryocyte Differentiation
Neutrophil Signaling
NK Signaling
Th17 Signaling
Th1-Th2 Signaling
Tissue Damage
Treg Signaling
Angiogenesis
ECM Degradation
Endothelial Innate Immune Activation
Fibrosis
Immune Regulation Of Tissue Repair
Wound Healing

Martin F, Sewer A, Talikka M, Xiang Y, Hoeng J, Peitsch MC (2014) Quantification of biological network perturbations for mechanistic insight and diagnostics using two-layer causal models. *BMC bioinformatics 15: 238*

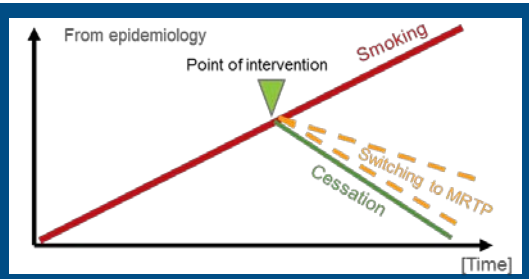
A case study of biomarkers of oxidative stress: ApoE mouse

ApoE mouse: Introduction and Objectives

Study: ApoE P1 RRP switching study

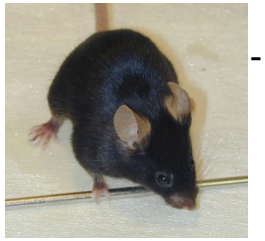
- **Main objective:**
  'Does switching from conventional 3R4F cigarettes to P1 MRTP [P1 RRP] halt or delay the progression of vascular and respiratory pathologies? If so, what are the cellular and molecular mechanisms affected by switching to P1 MRTP [P1 RRP] exposure and how similar are these mechanisms to smoking cessation?'

Plaque burden ~ CVD risk
Atherogenic lipids ~ CVD risk
Lung emphysema ~ COPD risk
Inflammation ~ x diseases risk



- **ApoE $^{-/-}$ mice (Taconics, USA)**

- **Comprehensive analysis of:**
  i) Cardiovascular endpoints
  ii) Emphysematous endpoints
  iii) Molecular endpoints (RNA, DNA, Protein, Lipids)



- **P1 RRP included as:**
  i) Chronic exposure (8 months)
  ii) Switching (2 months 3R4F, up to 6 months P1 RRP)

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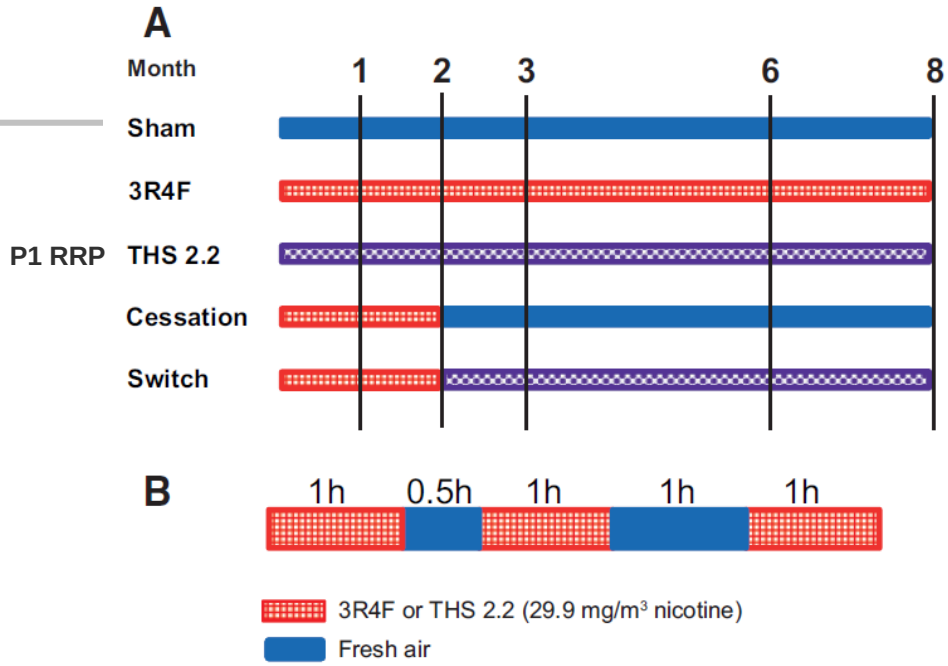
ApoE mouse: Study Design



OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 149(2), 2016, 441–457

doi: 10.1093/toxsci/kfv244
Advance Access Publication Date: November 17, 2015
Research Article

Effects of Cigarette Smoke, Cessation, and Switching to Two Heat-Not-Burn Tobacco Products on Lung Lipid Metabolism in C57BL/6 and *Apoe*$^{-/-}$ Mice—An Integrative Systems Toxicology Analysis

Bjoern Titz,*,[1] Stéphanie Boué,*,[1] Blaine Phillips,† Marja Talikka,* Terhi Vihervaara,‡ Thomas Schneider,* Catherine Nury,* Ashraf Elamin,* Emmanuel Guedj,* Michael J. Peck,* Walter K. Schlage,* Maciej Cabanski,*,[2] Patrice Leroy,* Gregory Vuillaume,* Florian Martin,* Nikolai V. Ivanov,* Emilija Veljkovic,* Kim Ekroos,‡ Reijo Laaksonen,‡ Patrick Vanscheeuwijck,* Manuel C. Peitsch,* and Julia Hoeng*,[3]

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TOXICOLOGICAL SCIENCES, 149(2), 2016, 411–432

doi: 10.1093/toxsci/kfv243
Advance Access Publication Date: November 25, 2015
Research Article

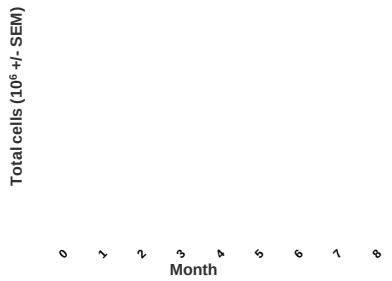
An 8-Month Systems Toxicology Inhalation/Cessation Study in Apoe$^{-/-}$ Mice to Investigate Cardiovascular and Respiratory Exposure Effects of a Candidate Modified Risk Tobacco Product, THS 2.2, Compared With Conventional Cigarettes

Blaine Phillips,* Emilija Veljkovic,† Stéphanie Boué,† Walter K. Schlage,‡ Gregory Vuillaume,† Florian Martin,† Bjoern Titz,† Patrice Leroy,† Ansgar Buettner,§ Ashraf Elamin,† Alberto Oviedo,* Maciej Cabanski,†,[1] Héctor De León,† Emmanuel Guedj,† Thomas Schneider,† Marja Talikka,† Nikolai V. Ivanov,† Patrick Vanscheeuwijck,† Manuel C. Peitsch,† and Julia Hoeng,†,[2]

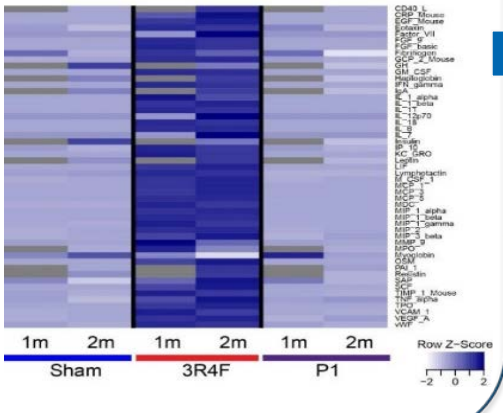
ApoE mouse: Potential for Reduced Risk

Lung Inflammation

Total lung cells

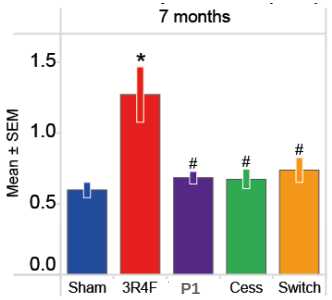


Mediators in bronchoalveolar lavage fluid



Cardiovascular

Aorta Plaque Volume



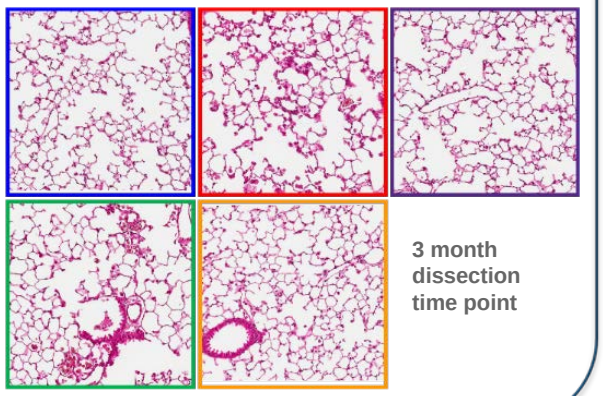
Lung function

Concomitant evaluation of CVD and COPD-related end points in ApoE-/- mice with RRP

Sham 3R4F P1 RRP

Cessation Switch

Lung histopathology



3 month dissection time point

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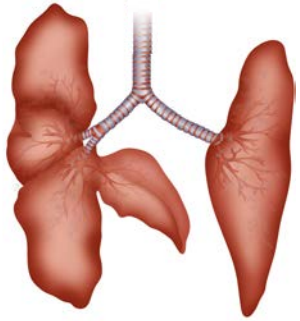
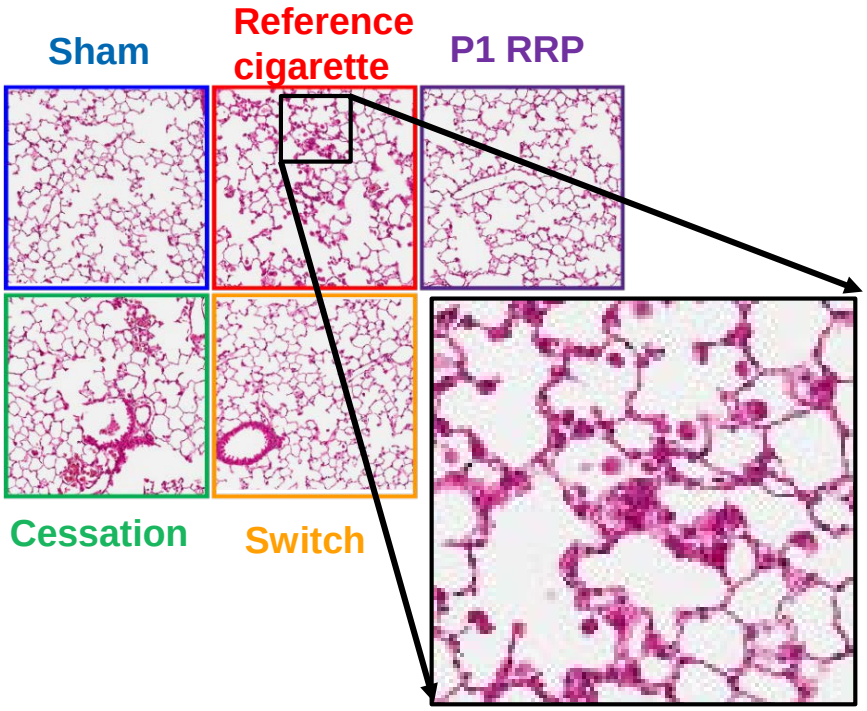
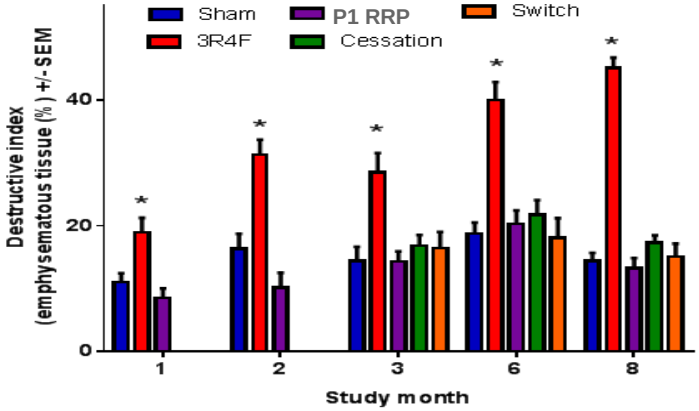
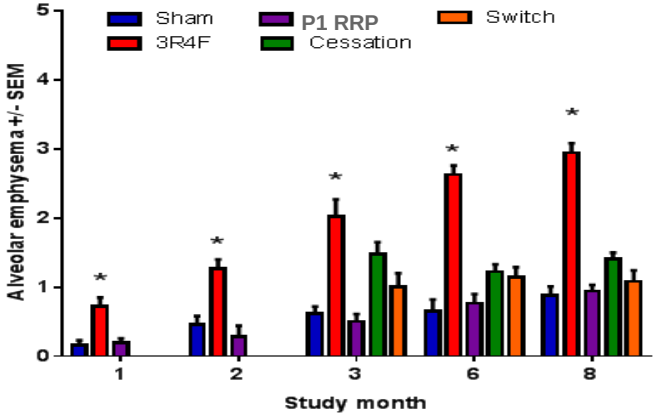
ApoE mouse: Respiratory Tract Histology









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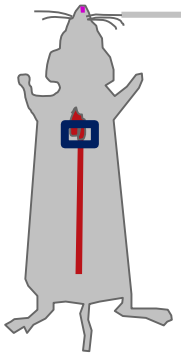
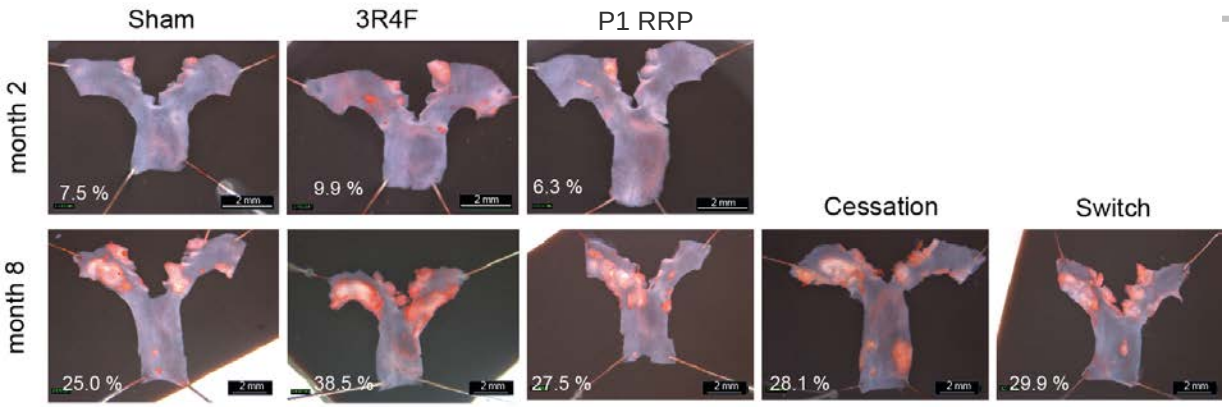
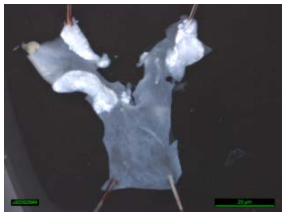
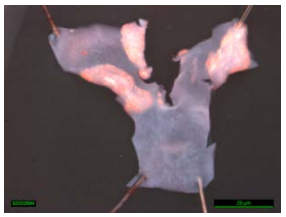
ApoE mouse: Semi-automated Analysis of the Aortic Arch for Plaque Areas







Unstained aortic arch



Stained aortic arch

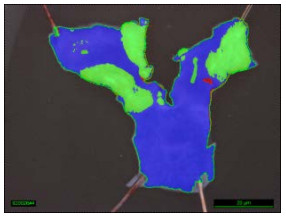
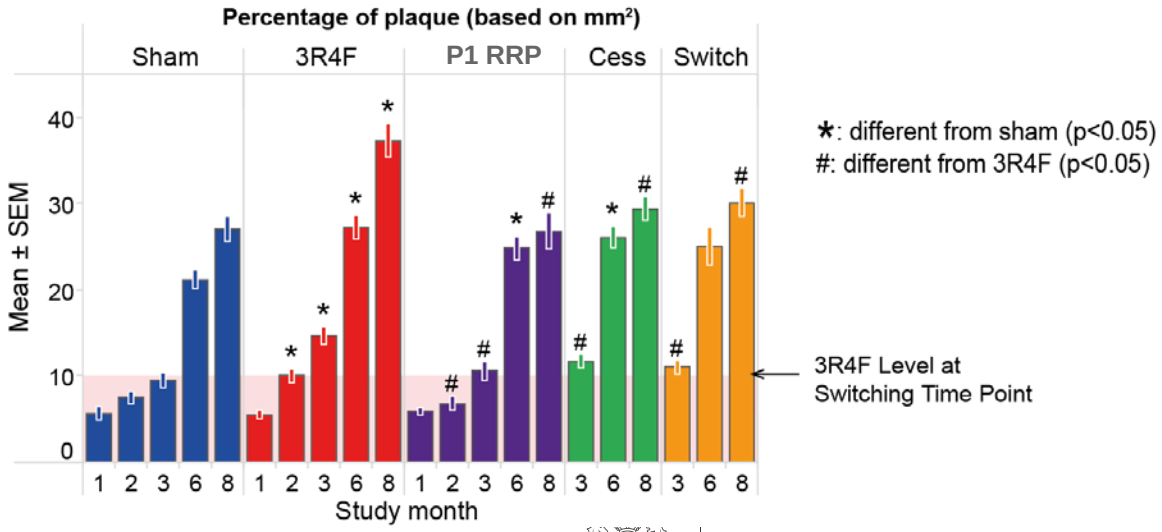
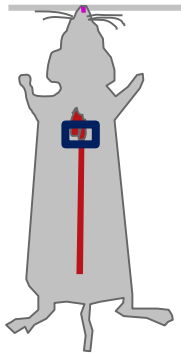


Image analysis Visiopharm

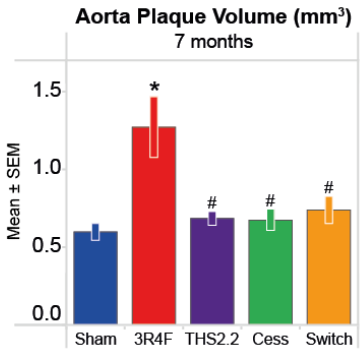


PMI RESEARCH & DEVELOPMENT

ApoE mouse: Measurement by microCT to Assess Plaque Volume



A.



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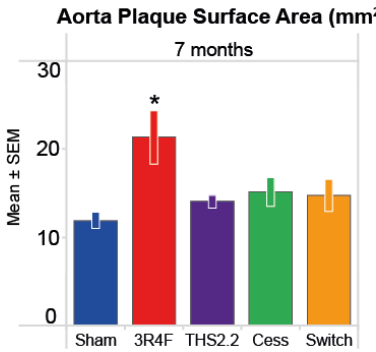
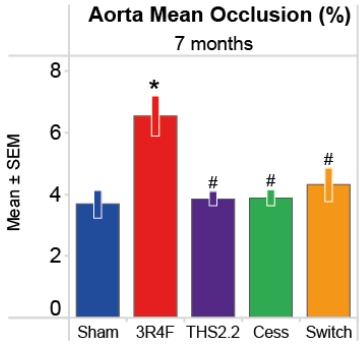
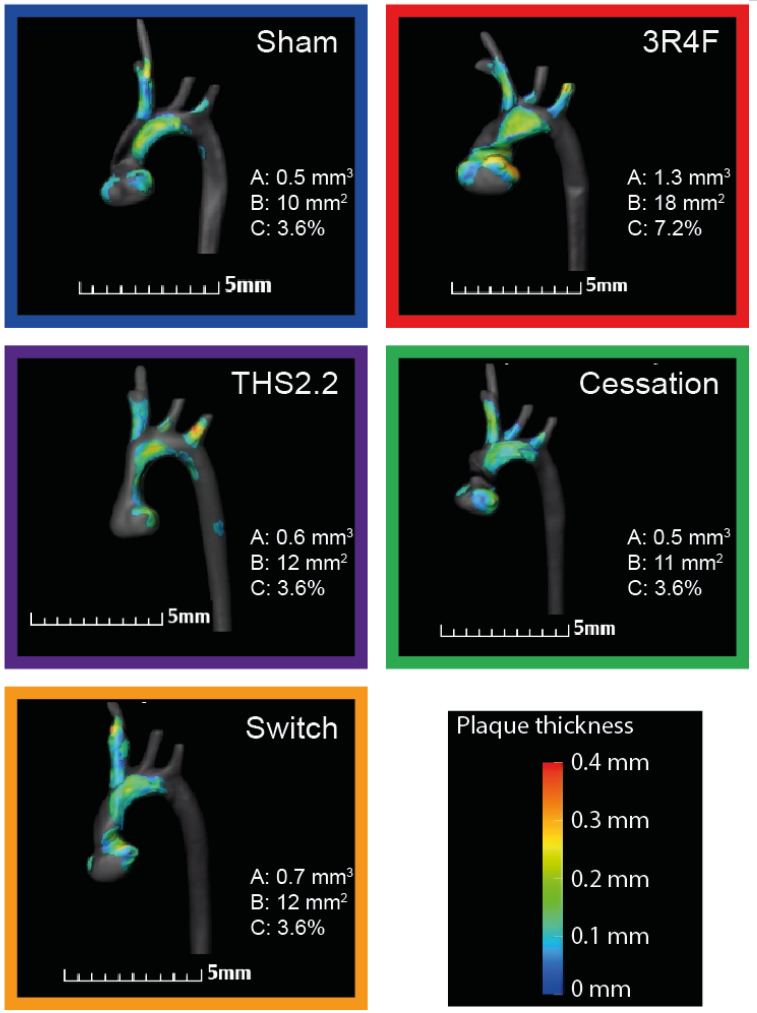


C.



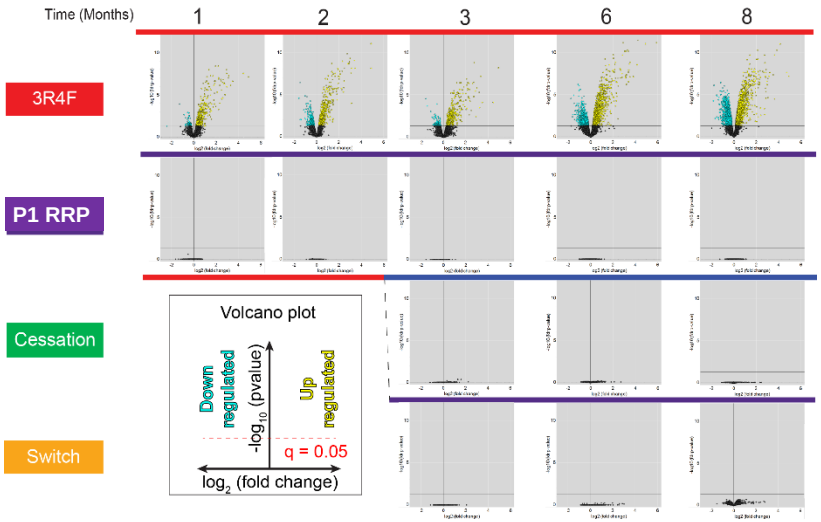
D.



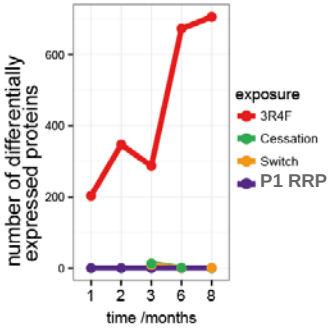
*: different from sham ($p<0.05$), #: different from 3R4F ($p<0.05$)

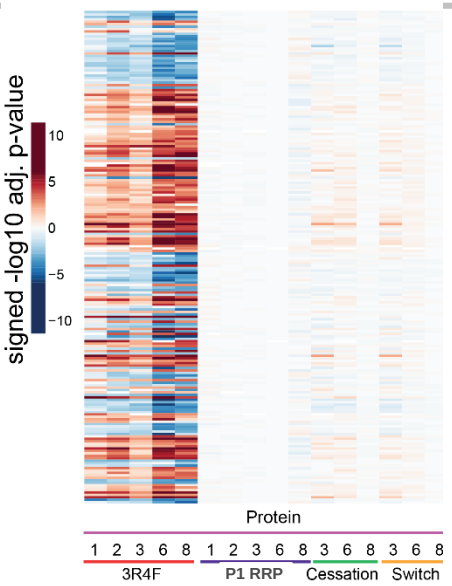
ApoE mouse: Lung Transcriptomics, Proteomics, and Lipidomics

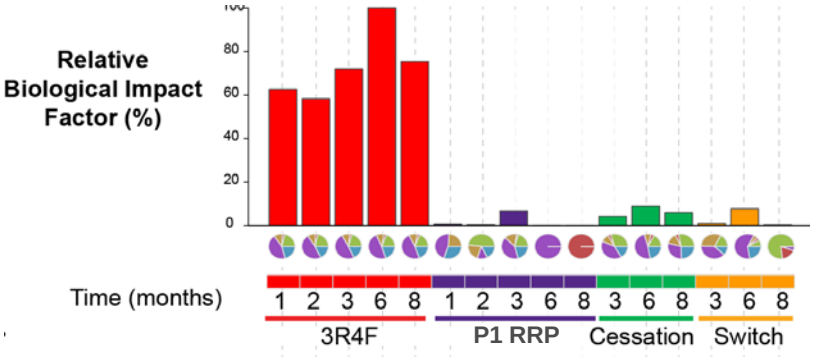
Transcriptomics



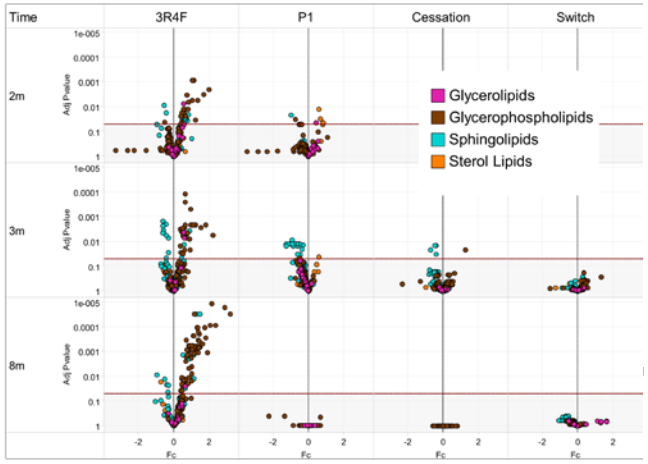
Proteomics



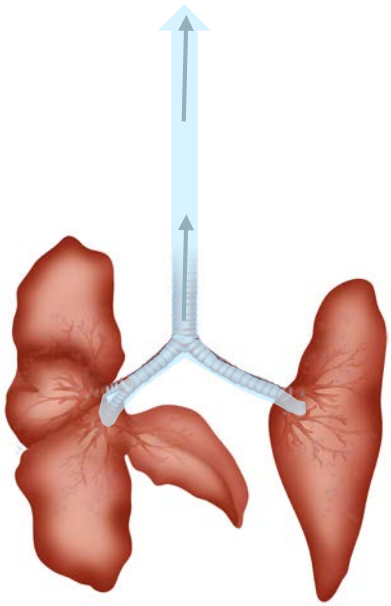


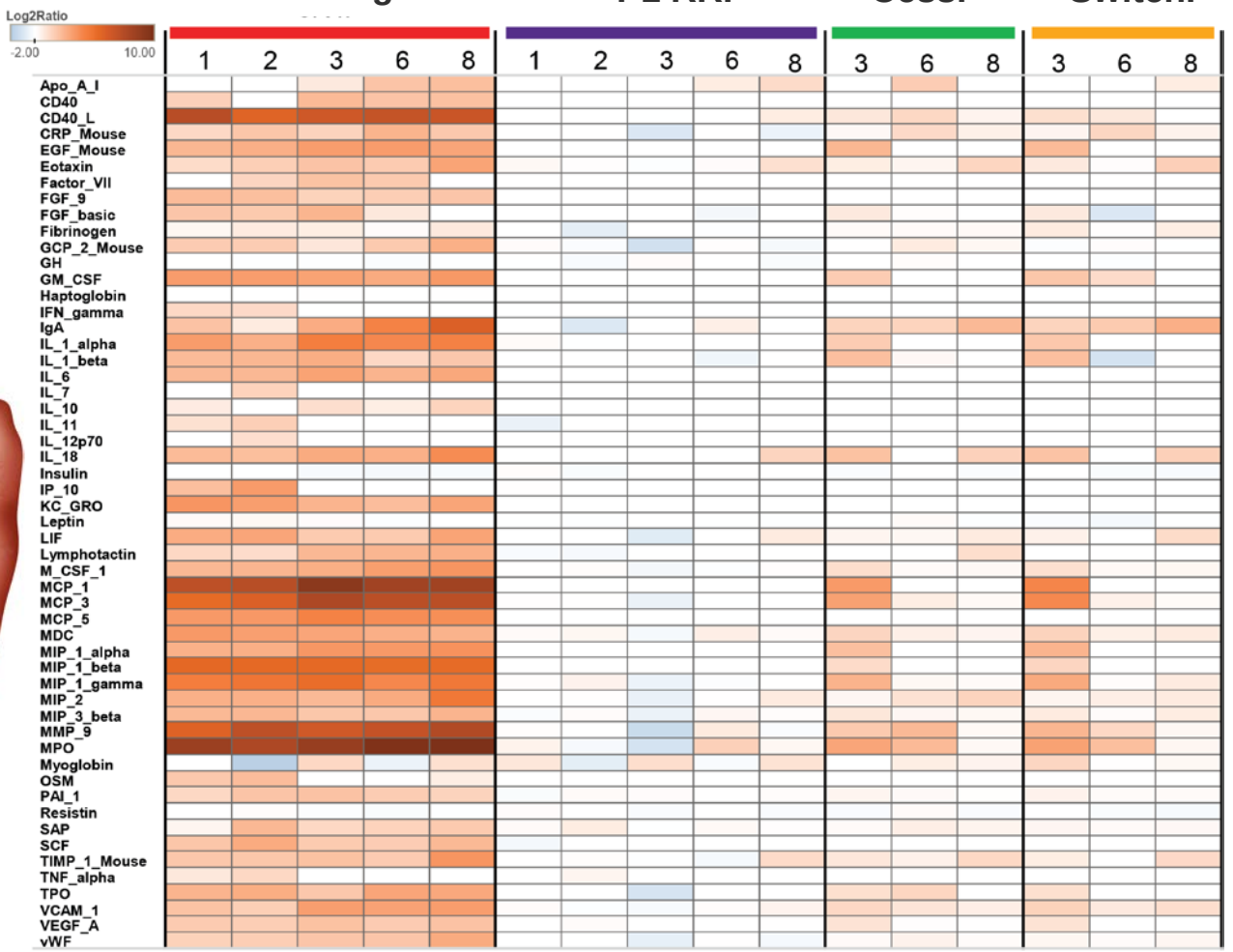


Lipidomics



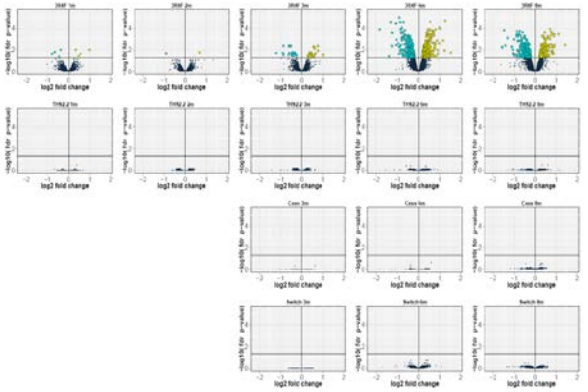
ApoE mouse: Inflammatory Mediators in Broncheoalveolar Lavage

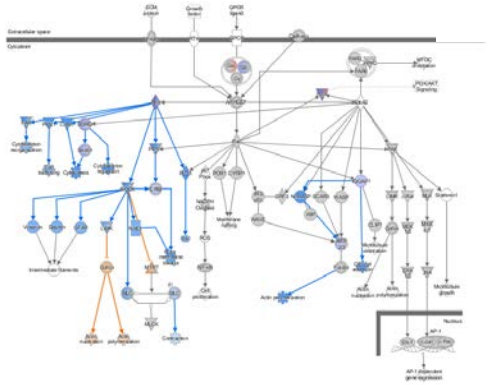


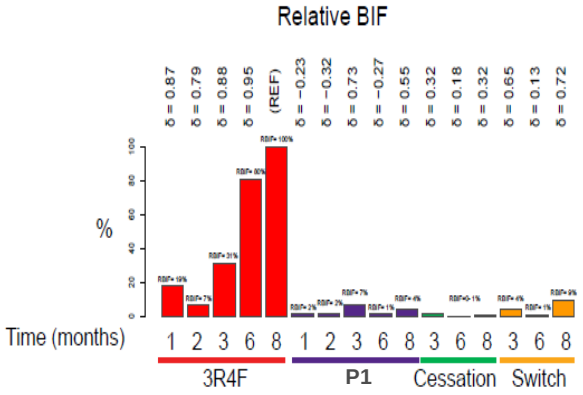


PMI RESEARCH & DEVELOPMENT

Heart Transcriptomics







The most important impact was observed in mouse heart exposed to 3R4F at 6 and 8 month time points.

P1 RRP, CESS and SWITCH groups didn't present any significant change

3R4F exposure significantly affected genes involved in cardiovascular function, such as cytoskeleton formation and structural components

Impact on actin and actinin expression suggest a strong effect of 3R4F on the contractile activity of muscle.

Quantify the impact of 3R4F exposure in comparison to P1 RRP, CESS and SWITCH and showed a highly reduced impact of P1 RRP exposure.

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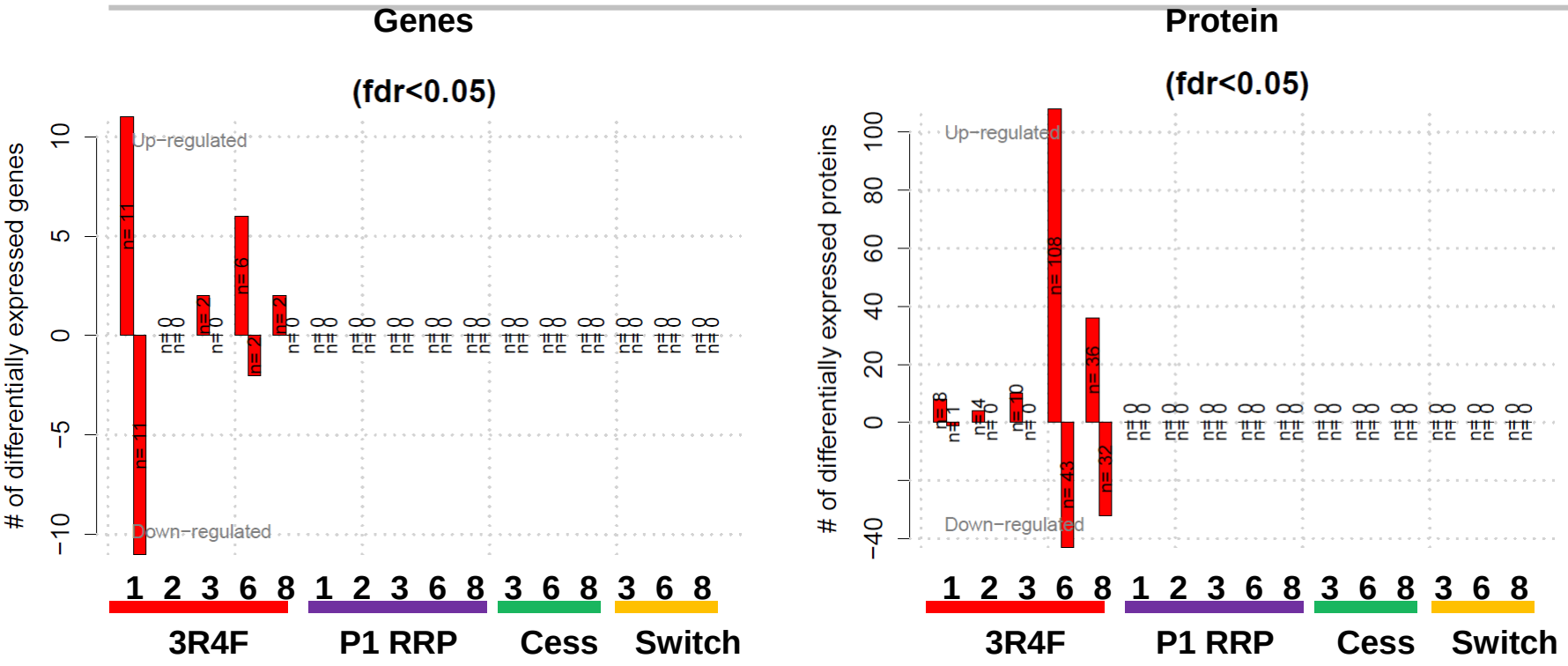
Liver Transcriptomics and Proteomics

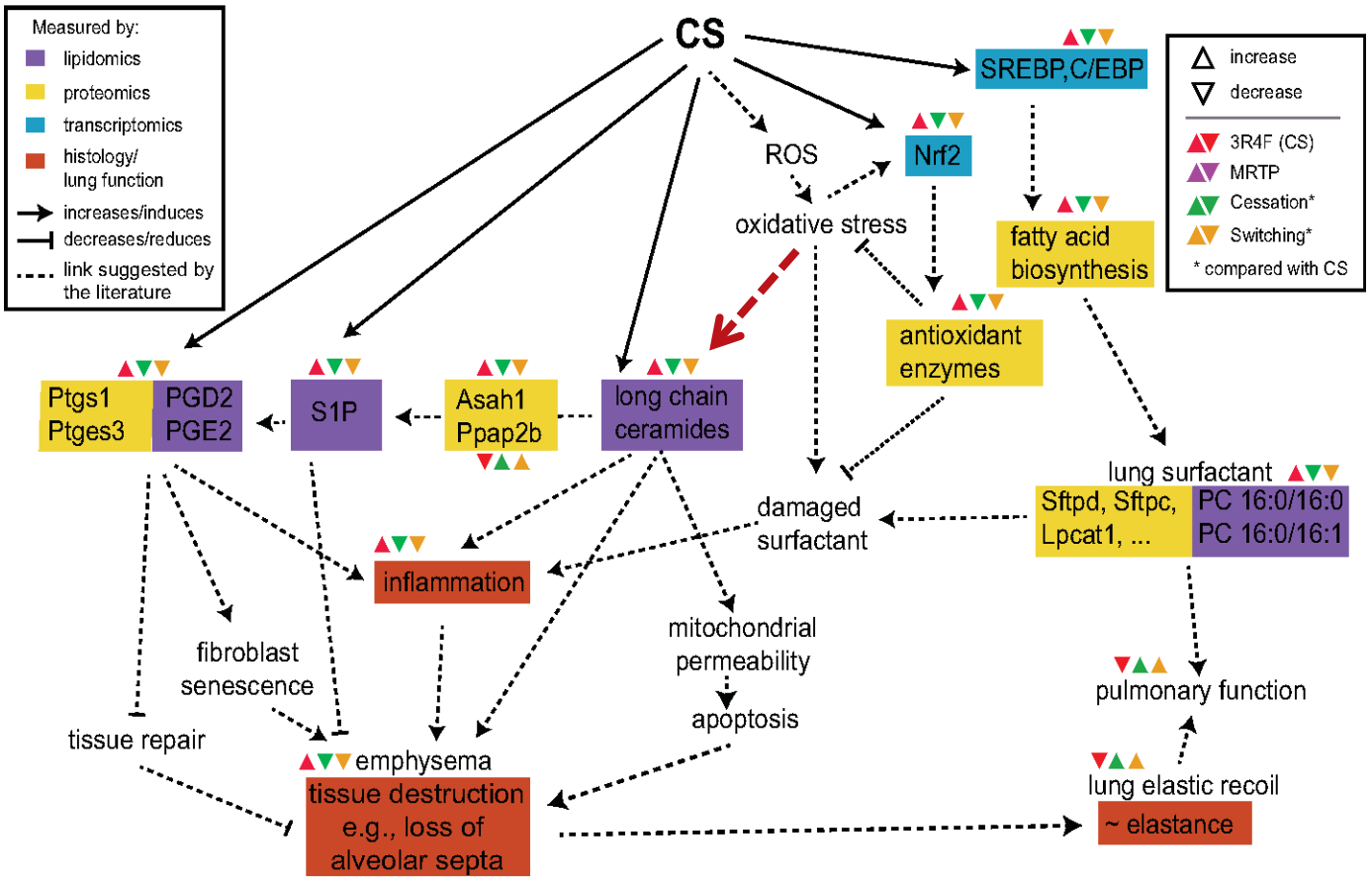


- **3R4F smoke or P1 RRP aerosol exposures do not cause macroscopic evidences of hepatotoxicity although molecular effects are appreciable.**
- **Xenobiotic and lipid metabolism-related genes/proteins are the most profoundly affected by 3R4F, but not by P1 RRP, Switch, or Cessation.**

PMI RESEARCH & DEVELOPMENT

Working hypothesis: Interplay among lipids and proteins in cigarette smoke (CS)-induced lung damage

Figure 7



Titz et al Toxicol Sci. 2015 Nov 17. pii: kfv244.

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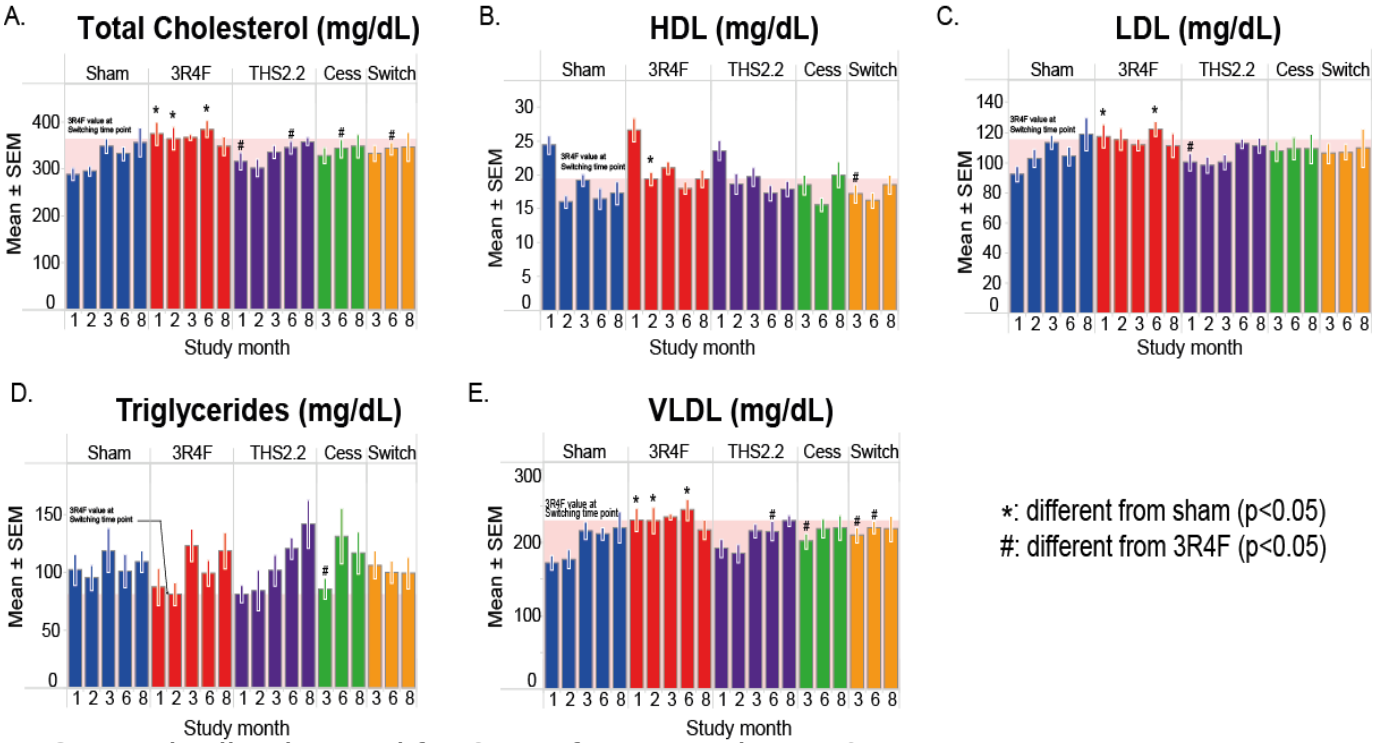
ApoE mouse: Plasma Lipid Profile



*: different from sham (p<0.05)
#: different from 3R4F (p<0.05)

TC marginally elevated for 3R4F from month 1 to 6
HDL inconsistently elevated for 3R4F at months 1 to 3
LDL inconsistently elevated for 3R4F at months 1 and 6
TG inconsistently elevated for 3R4F at months 1 and 3
VLDL significantly increased in 3R4F CS-exposed mice at months 1, 2, and 6, with the greatest difference to sham seen at month 1.

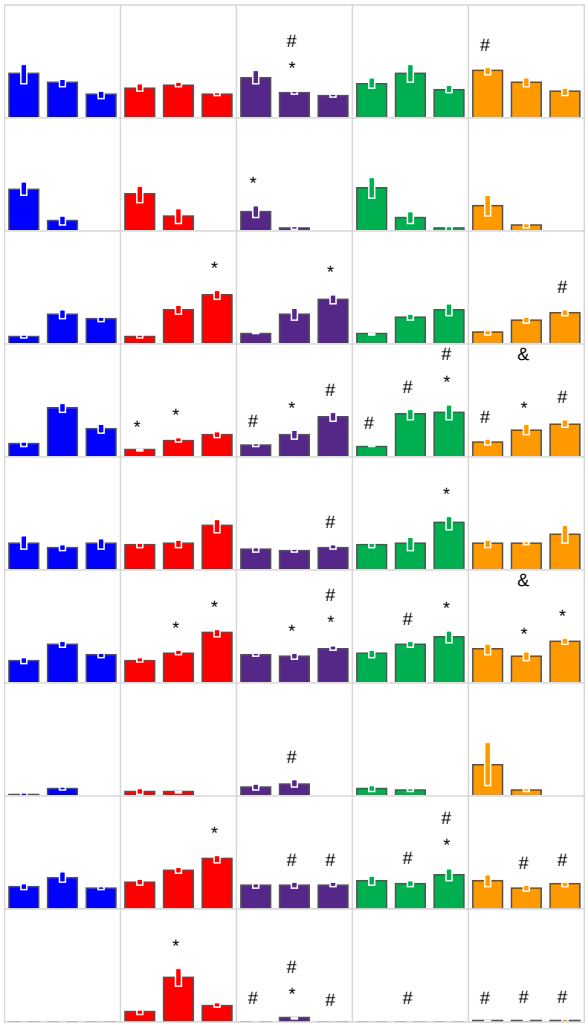
PMI RESEARCH & DEVELOPMENT

ApoE mouse: Oxidative Stress Biomarkers

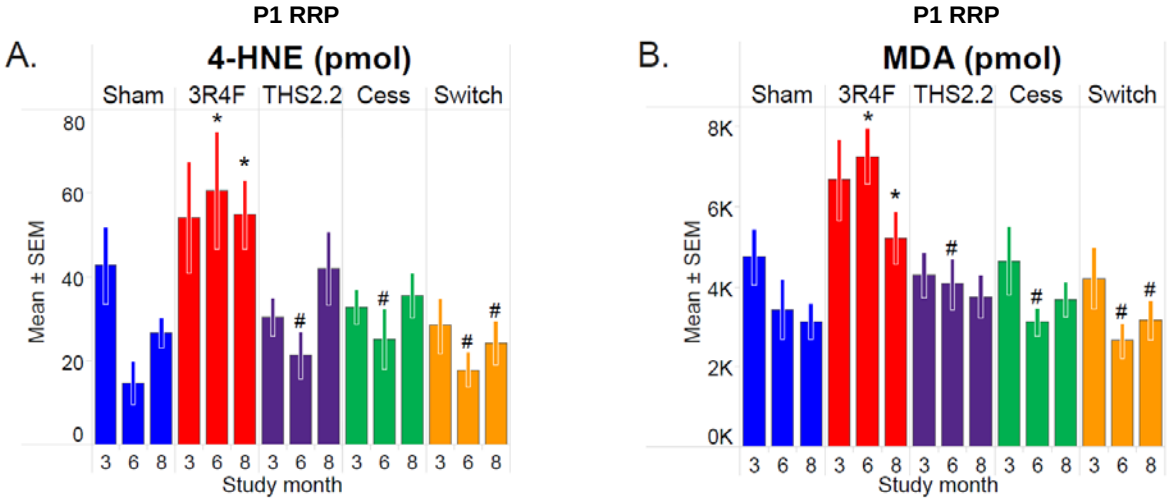
Biomarkers measured in urine (of individual mice) at 3, 6, and 8 months of exposure –
Apoe-/- P1 RRP study

A general disadvantage of urinary biomarkers in mice is **the difficulty to obtain reproducibly a significant amount of urine** that is representative of the metabolic processes during the collection period (usually 24 h, in inhalation studies often 18 h after end of exposure to avoid contamination with substances from the inhalation atmosphere).

***: different from sham (p<0.05)**
#: different from 3R4F (p<0.05)
&: different from Cessation (p<0.05)



ApoE study: Urinary Biomarkers of Oxidative stress and inflammation



4-hydroxynonenal

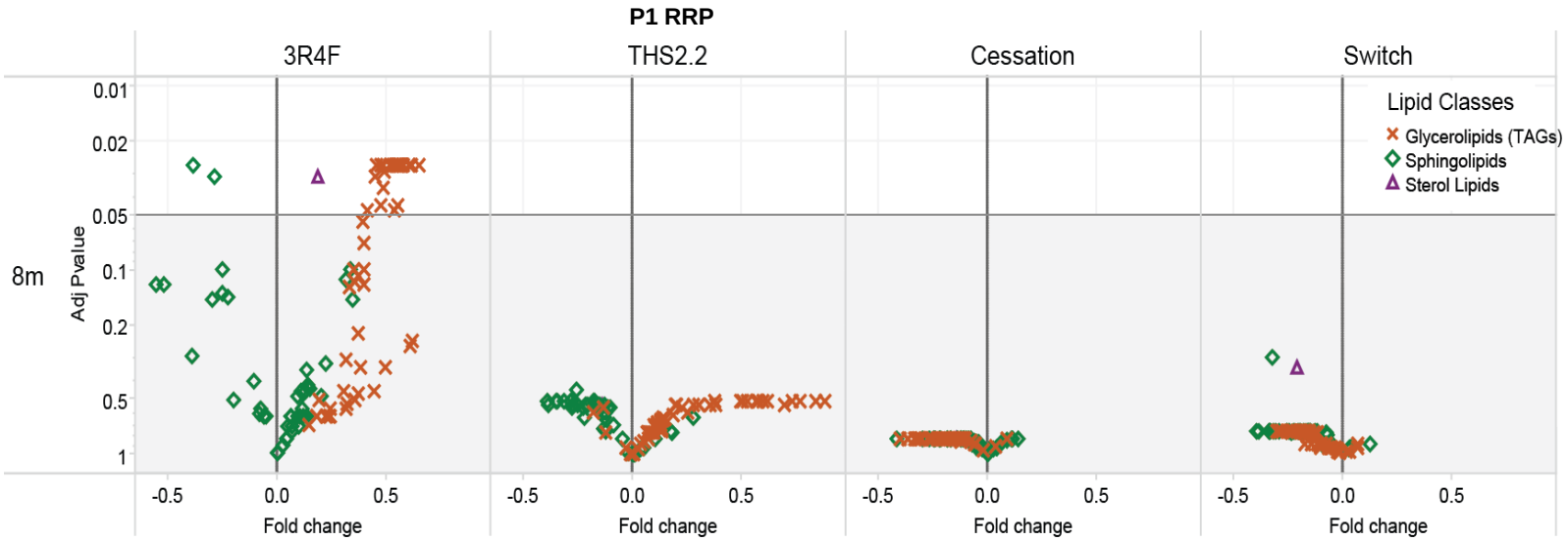
malondialdehyde

However, the urinary 4-HNE and MDA values in the 3R4F groups had a consistent **trend to be higher than the sham values**, and the differences were statistically significant at months 6 and 8. that. The cessation, THS2.2 [P1 RRP], and switching groups were not different from the sham controls.

Urine was collected throughout an 18-hour post-exposure period, and the respective metabolites were determined by GC-NCI-MS (A, B) or UPLC-MS/MS (C). A. 4-HNE, B. MDA. Mean ± SEM, n = 8. P-values against Sham obtained by t-test accounting for variance heterogeneity (*) or by Wilcoxon exact MC (+). */+++p < 0.001; **/++p < 0.01; */+p < 0.05**

PMI RESEARCH & DEVELOPMENT

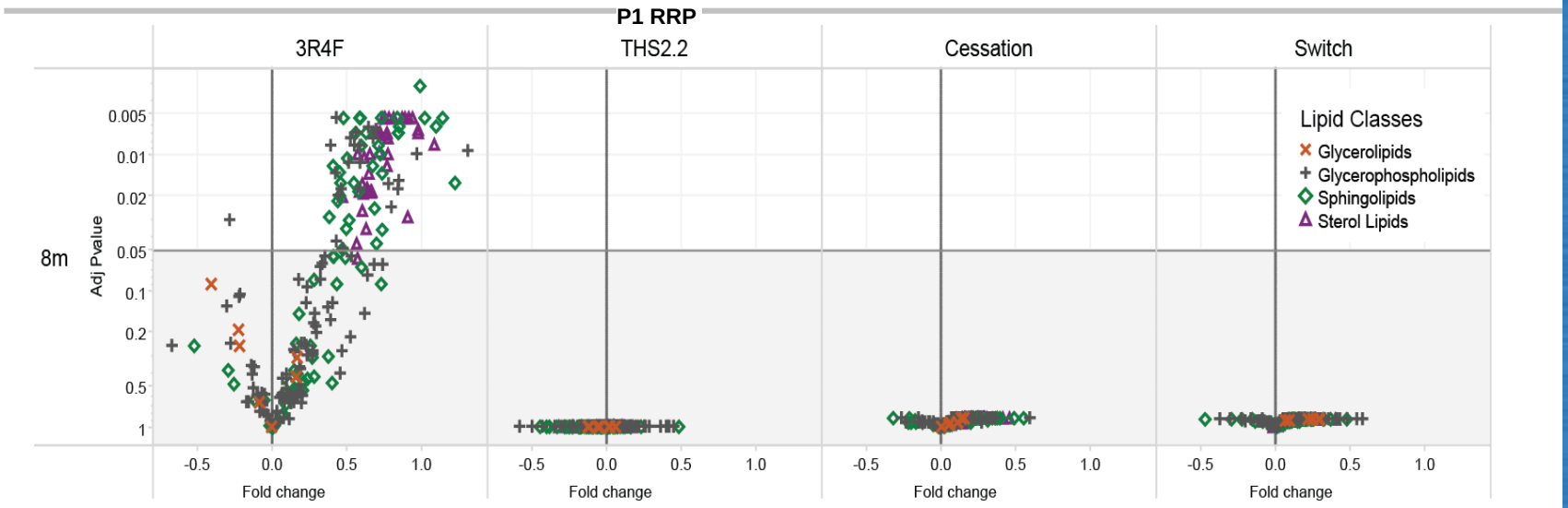
ApoE mouse: Plasma Lipidomics (vs. Sham 8 month)

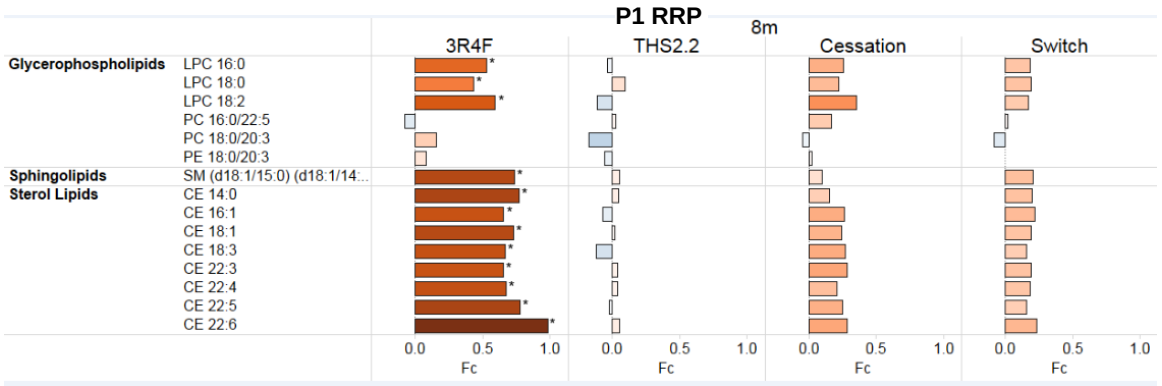


Only in the 3R4F group **several TAG species** displayed a statistically significantly **higher level** than in the sham, and some **sphingolipids** showed significant **decreases**.. Two individual ceramides, LacCer(d18:1/18:0) and Gb3(d18:1/24:0), were lower in the 3R4F-exposed mice It is noteworthy that the plasma lipidome of mice from the cessation, THS2.2 [P1 RRP], and switching groups did not exhibit significant changes. The decreases in several ceramides/sphingolipids at the 8-month time-point in the 3R4F group are resembling some of our previously observed decreases at the 6-month time-point.

PMI RESEARCH & DEVELOPMENT

ApoE mouse: Aortic Arch Lipidomics





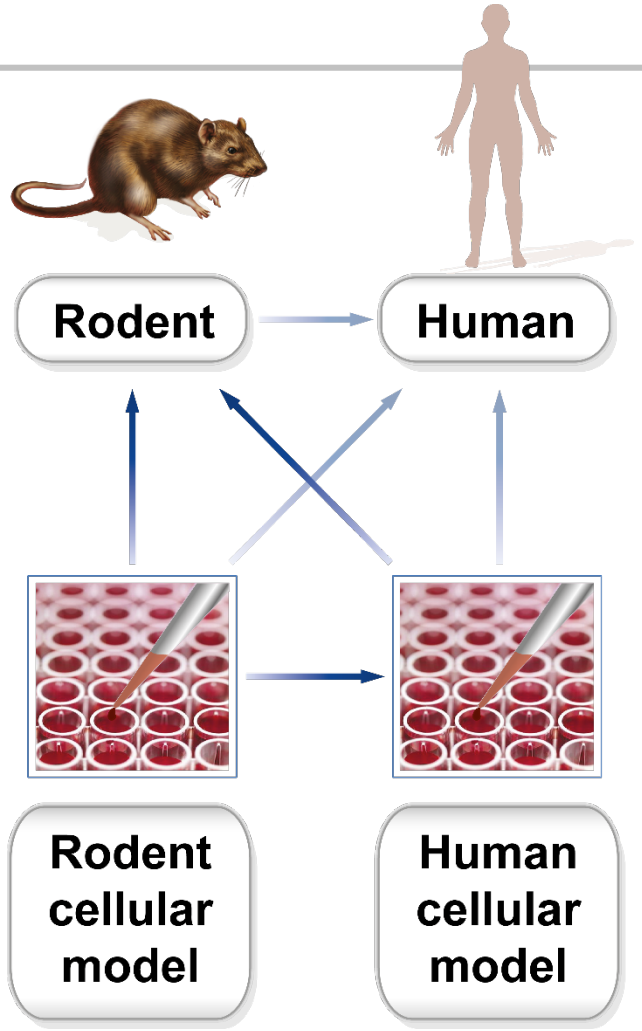
Aortic arch lipids from exposed ApoE mice vs sham controls shared with **the human plaque-enriched lipids** reported by Stegemann et al., (2011) Circ Cardiovasc Genet. Jun;4(3):232-42.

PMI RESEARCH & DEVELOPMENT

ApoE mouse: Conclusions

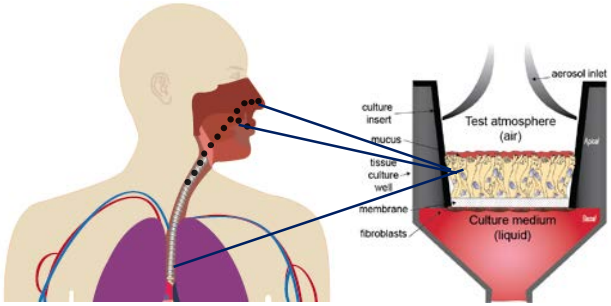
- ApoE -/- mouse is a suitable model system to study both cardiovascular and COPD endpoints
- Lipidomics, transcriptomics and proteomics seem to be promising approaches to identify novel oxidative stress biomarkers.
- Chronic exposure to P1 RRP shows a limited effect across all parameters relative to 3R4F exposure. In most cases analyzed so far – results are approaching those of the sham-exposed animals.

Translation between species and experimental systems



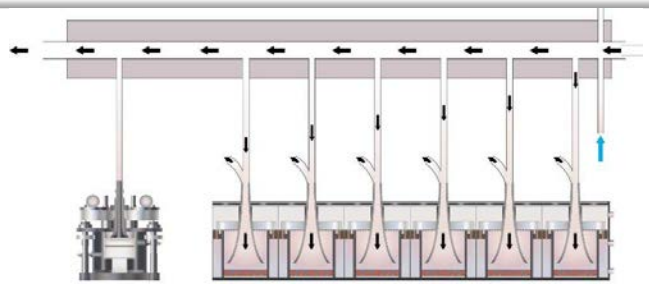
Poussin C, Mathis C, Alexopoulos LG, Messinis DE, Dulize RHJ, Belcastro V, Melas IN, Sakellaropoulos T, Rhrissorrakrai K, Bilal E, Meyer P, Talikka M, Boue S, Norel R, Rice JJ, Stolovitzky G, Ivanov NV, Peitsch MC and Hoeng J (2014) The Species Translation Challenge - A Systems Biology Perspective on Human and Rat Bronchial Epithelial Cells. Scientific Data, 1:140009

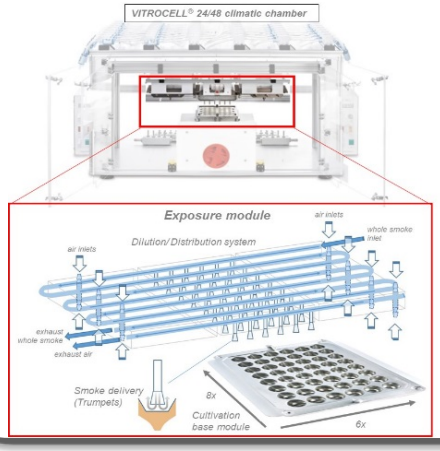
In-Vitro Whole Smoke Exposure Experiments



Organotypic respiratory tissue cultures can be cultured at the air-liquid interface

The smoking machine produces aerosol from tobacco products





In the climatic chamber with an exposure module, up to 48 wells can be exposed simultaneously. The base module has a format of 8 rows x 6 columns.

PMI RESEARCH & DEVELOPMENT

QASMC: Biomarker Identification Clinical Study

(QASMC is Queen Ann Street Medical Centre)

QASMC Study: Objective

Primary Objective

To identify a biomarker/panel of biomarkers for the differentiation of subjects with COPD, current smokers, ex-smokers and never smokers (who have never smoked) using gene and protein analyses in biological samples.

Secondary Objectives

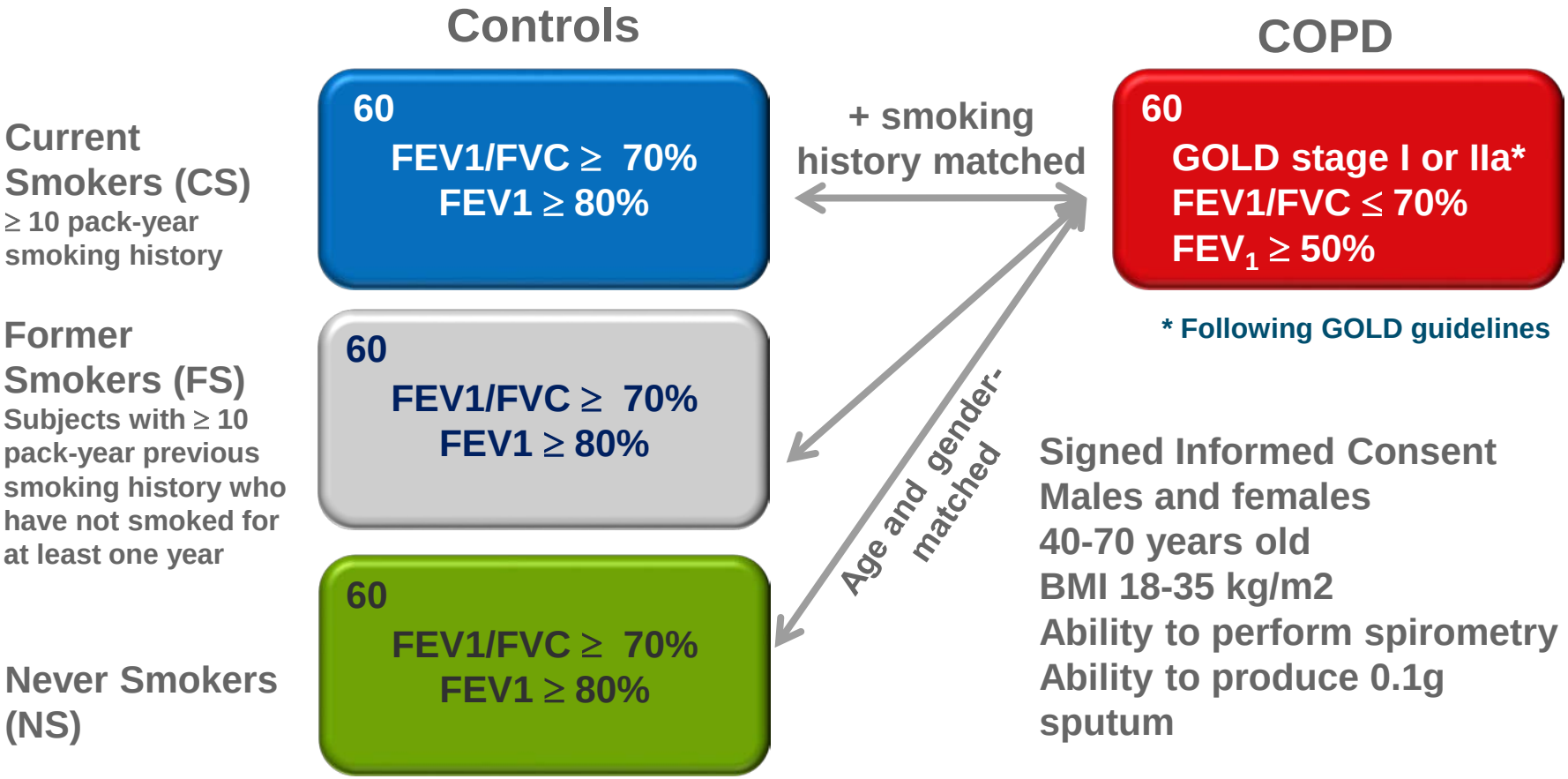
1) To determine optimal methods of biomarker analysis in sputum, nasal and blood samples.

2) To compare physiological measurements in subjects with COPD, smokers, ex-smokers and never smokers.

3) To compare quality of life in subjects with COPD, smokers, ex-smokers and never smokers.

QASMC Study: Design

Non-interventional, observational case-control design study conducted in the United Kingdom, and approved by the UK National Health Service (NHS) Ethics Committee



Signed Informed Consent
Males and females
40-70 years old
BMI 18-35 kg/m2
Ability to perform spirometry
Ability to produce 0.1g sputum

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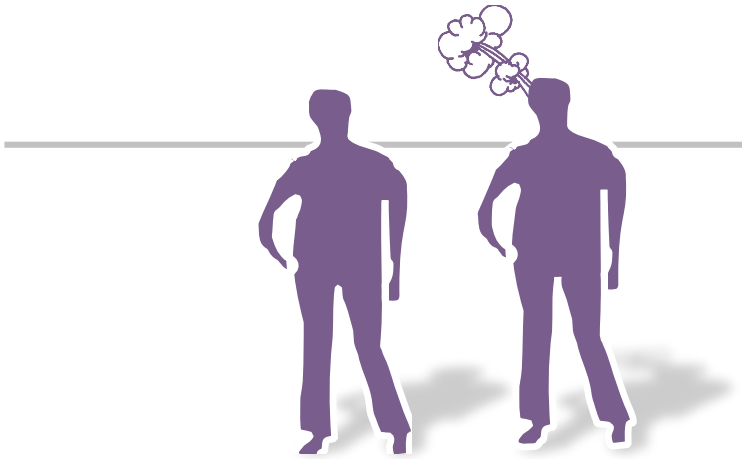
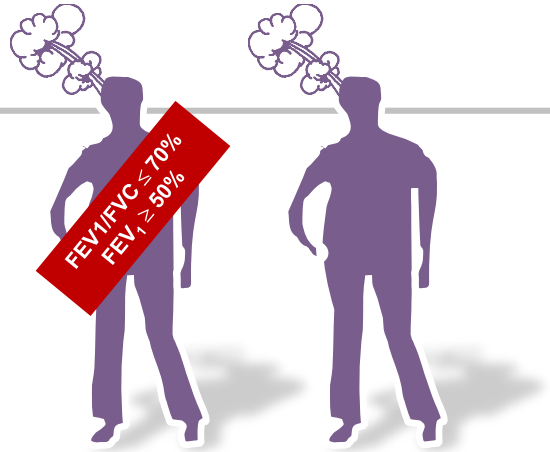
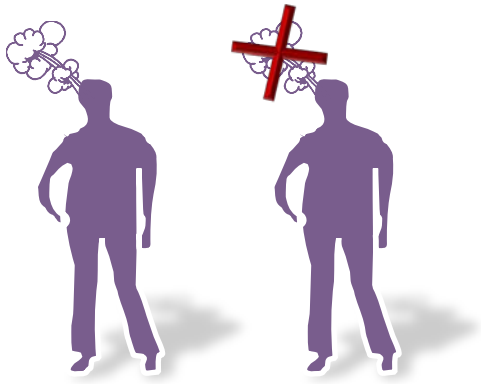
QASMC study: Overarching Scientific Questions



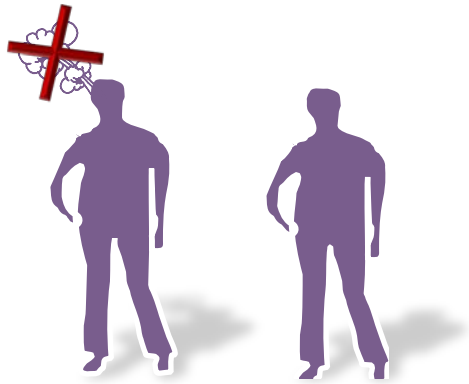
What is the biological impact of smoking?



What is the COPD specific biology in smokers?



Which of the smoking effects are reversible?



Which of the smoking effects are irreversible?

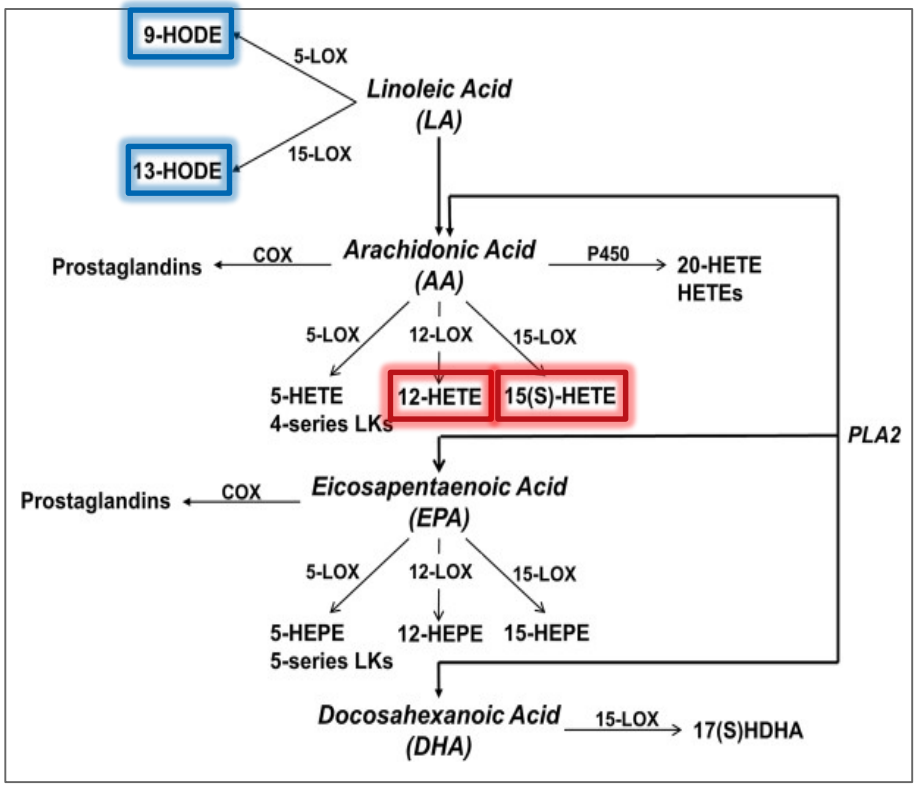
PMI RESEARCH & DEVELOPMENT

QASMC study: Description of Study Population

	NS	FS	CS	COPD	p-value
N	40	40	40	40	
Age (years)	56.00	57.03	55.68	58.05	0.4031
Gender (M/F)	22/18	22/18	22/18	22/18	1.0000
BMI	26.75	27.46	27.38	26.53	0.5890
Pack-years	-	27.61	34.48	45.71	<0.0001
FEV_1 (% pred)	112.5%	112.0%	103.4%	81.0%	<0.0001
FEV_1/FVC	79.6%	77.8%	75.2%	58.9%	<0.0001

QASMC study: Why Study Eicosanoid Metabolism?

- DHETs have been implicated in regulating vascular tone[1] and monocyte chemotaxis in response to MCP-1 *via* activation of PLA2 [2]
- HODEs are oxidation products of LA involved in monocyte maturation and macrophage gene expression during PPARγ-mediated atherogenesis[3]
- Increased levels of DHETs and decreased levels of HODEs suggest enhanced AA metabolism and activation of PLA2-mediated inflammatory pathways, possibly bypassing PPARγ-mediated inflammation



Modified from: Klawitter *et al. BMC Nephrology* 2013 14:165

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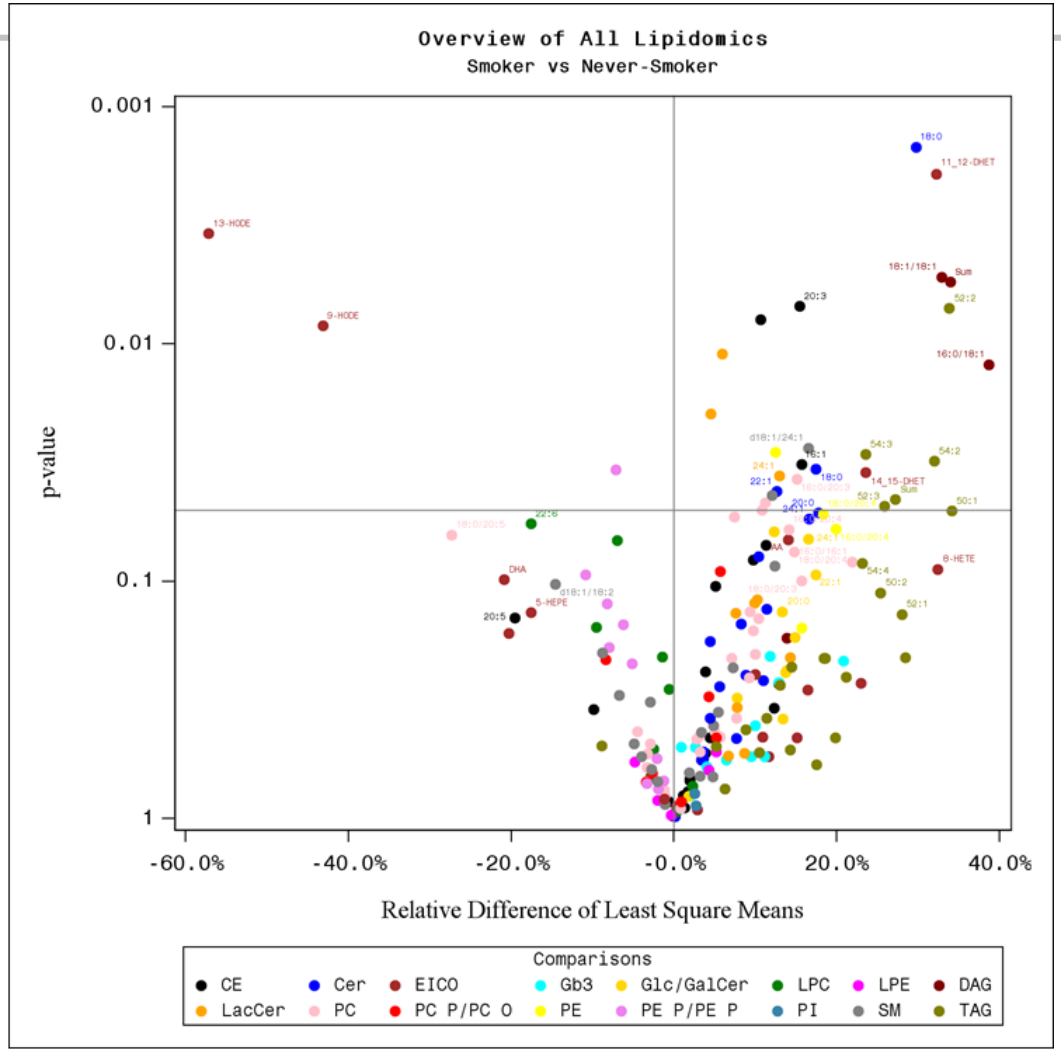
1) Weintraub et al. Circ Res. 1997 Aug;81(2):258-67. 2) Kundu et al. J Lipid Res. 2013 Feb;54(2):436-47. 3) Nagy et al. Cell. 1998 Apr 17;93(2):229-40.

QASMC study: The Effects of Smoking on the Serum Lipidome

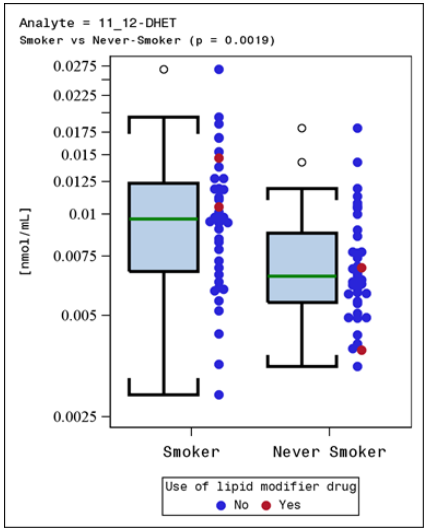
- Overall, levels of total diacyl- and triacylglycerols, lactosylceramides, phosphatidylcholines and -ethanolamines were significantly higher in the serum of CS than NS
- Some specific eicosanoids, cholesteryl esters, ceramides and sphingomyelins were also **significantly altered** in serum from CS compared to NS

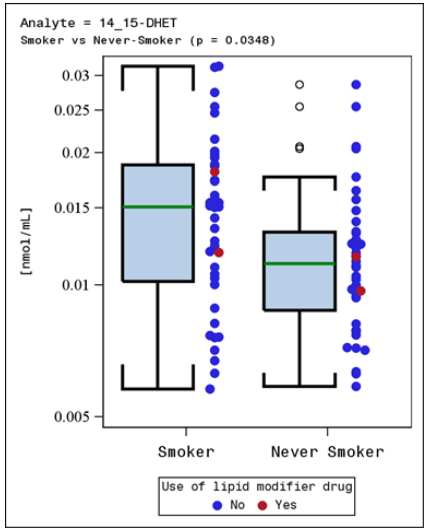


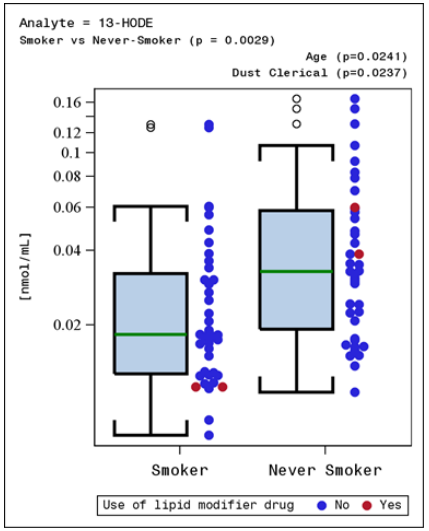
PMI RESEARCH & DEVELOPMENT

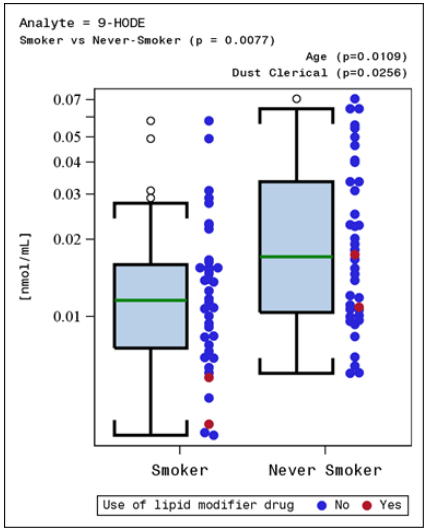
QASMC study: Serum Eicosanoids CS vs NS

- Compared to never-smokers, smokers had significantly higher levels of serum arachidonic acid metabolites 11,12-DHET and 14,15-DHET
- Smokers also had significantly lower levels of serum linoleic acid metabolites 13-HODE and 9-HODE than never-smokers





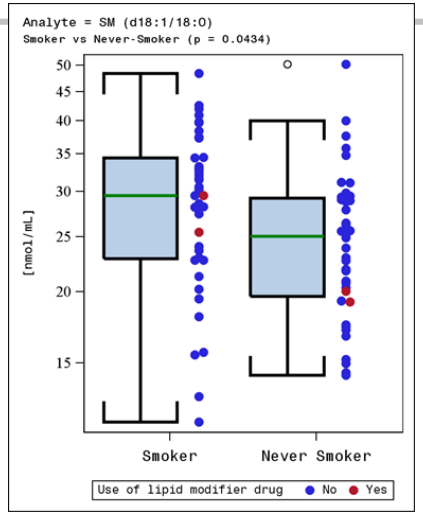


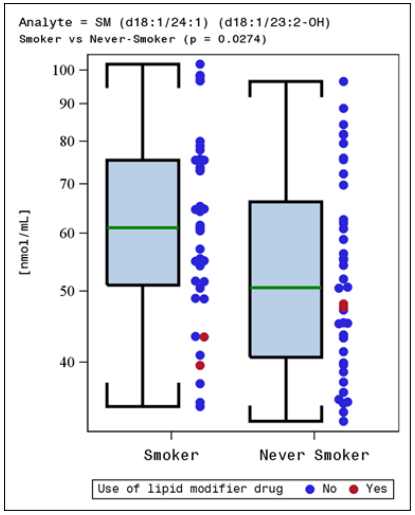


PMI RESEARCH & DEVELOPMENT

QASMC study: Serum Sphingomyelin (SM) CS vs NS

- Compared to never-smokers, smokers had significantly higher levels of serum SM(d18:1/18:0) and SM(d18:1/24:1)
- Sphingomyelins are ubiquitous in all cell membranes and involved in signal transduction, membrane trafficking and protein sorting[4]
- Increased plasma SM levels have been associated with sub-clinical atherosclerosis and an increased risk for serious cardiovascular events[5]
- Sphingomyelin is also a part of the pulmonary surfactant[6] and levels in BALF and IS from “healthy” smokers are increased compared to non-smokers7
- Elevated SM levels could be reflective of active/enhanced cellular breakdown in response to cigarette smoking





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4) Brown et al. J Biol Chem. 2000 Jun 9;275(23):17221-4.
5) Nelson et al. Am J Epidemiol. 2006 May 15;163(10):903-12.
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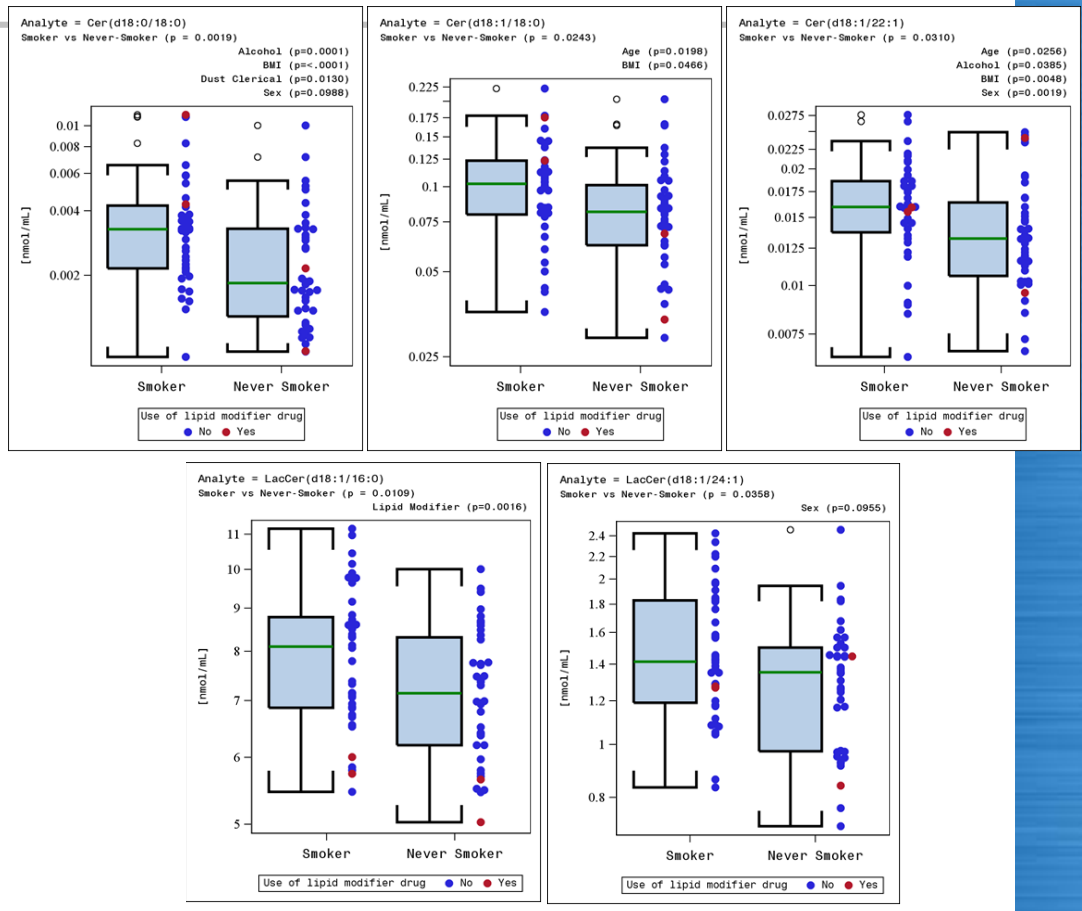
QASMC study: Serum Ceramides CS vs NS

- Compared to never-smokers, smokers had significantly higher levels of serum Cer(d18:0/18:0), Cer(d18:1/18:0), Cer(d18:1/22:1), LacCer(d18:1/16:0), and LacCer(d18:1/24:1)
- Ceramides are key intermediates in the biosynthesis of all complex sphingolipids and function as 2nd messengers in a variety of biological processes[8]
- Increased serum ceramide levels have been linked to oxidative stress and inflammation[9,10] and could be linked to early lung damage[11]



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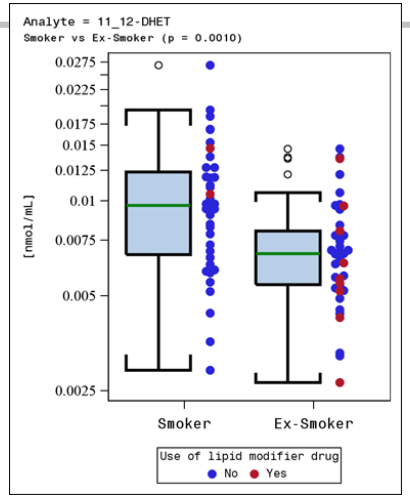
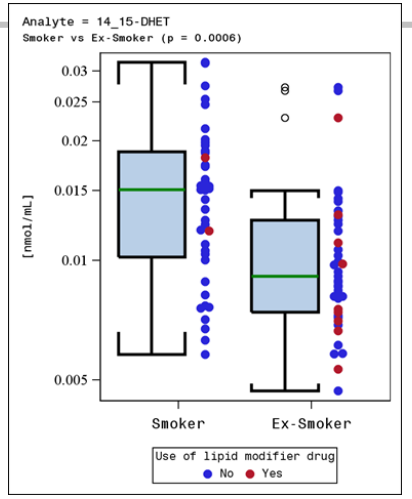
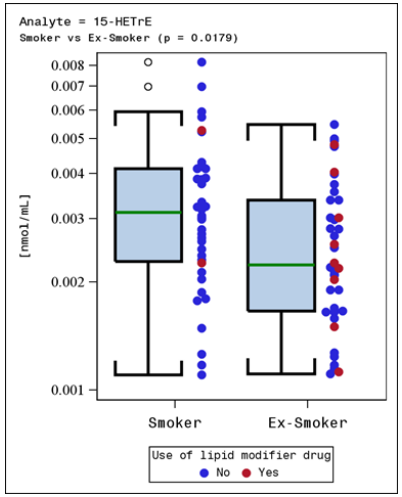
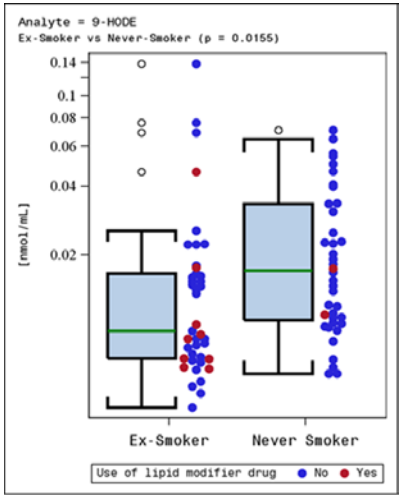
QASMC study: Serum Eicosanoids CS vs FS

- Compared to former smokers, smokers had significantly higher levels of serum 11,12-DHET, 14,15-DHET, and 15-HETrE
- In addition, 9-HODE serum levels were significantly lower in former smokers than never-smokers.
- Cigarette smoking is linked to increased levels of the AA metabolites 11,12-DHET and 14,15-DHET and decreased LA metabolite levels (see CS vs NS comparison), and this event appears to be only partially reversible









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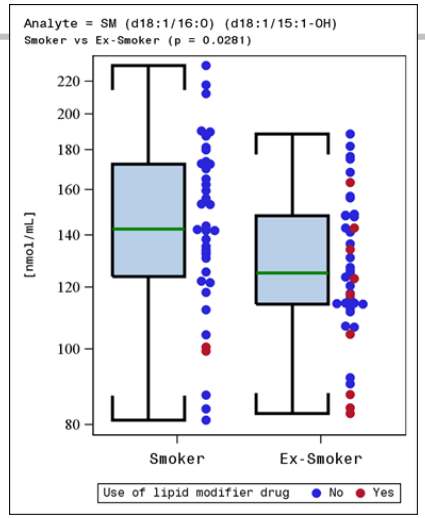
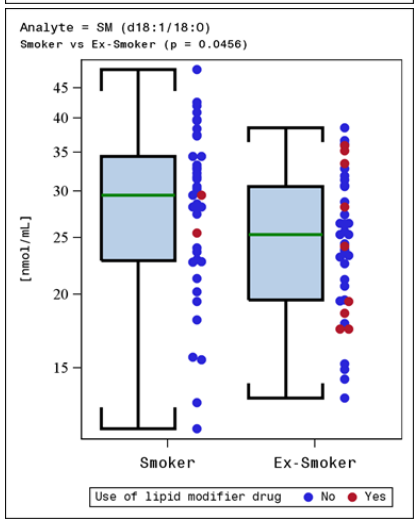
QASMC study: Serum Sphingomyelins CS vs FS

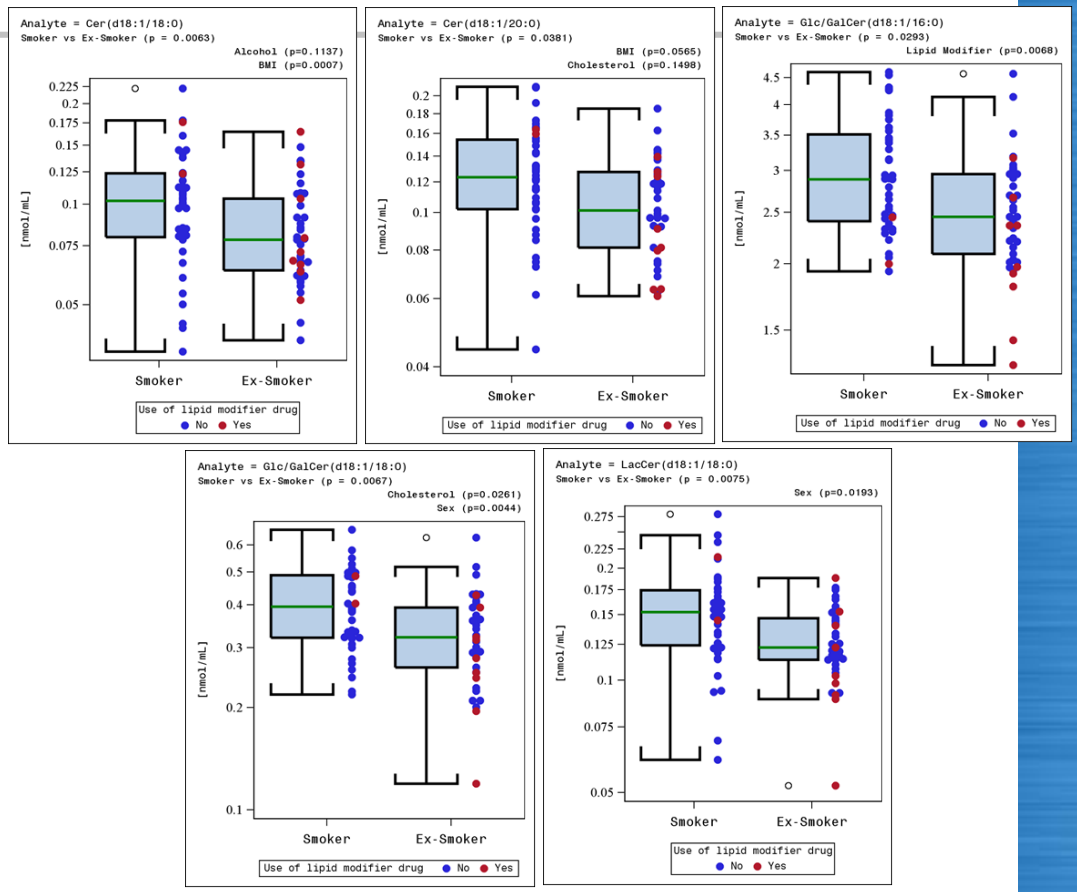
- Compared to former smokers, smokers had significantly higher levels of serum SM(d18:1/16:0) and SM(d18:1/18:0)
- One of these SM was also elevated in serum from smokers compared to never-smokers.





QASMC study: Serum Ceramides SM vs FS

- Compared to former smokers, smokers had significantly higher levels of serum Cer(d18:1/18:0), Cer(d18:1/20:0), Glc/GalCer(d18:1/16:0), Glc/GalCer(d18:1/18:0), and LacCer(d18:1/18:0)
- One of the altered ceramide molecules, Cer(d18:1/18:0), was also seen to be different between smokers and never-smokers.
- Additionally, there are some simple glycosphingolipid molecules that appear to differentiate smokers from former smokers. These intermediates in sphingolipid metabolism have been linked to various immune functions including T cell signaling.
- Lower serum levels in former smokers could be indicative of subsiding smoking-related systemic inflammation.



QASMC Study: Overview of the Lipidomics Endpoints Analyzed in Serum

	CS vs NS	FS vs NS	CS vs FS	COPD vs CS
11,12-DHET	↑	≈	↑	≈
14,15-DHET	↑	≈	↑	≈
13-HODE	↓	≈	≈	≈
9-HODE	↓	↓	≈	≈
SM(d18:1/16:0)	≈	≈	↑	↓
SM(d18:1/18:0)	↑	≈	↑	↓
SM(d18:1/24:0)	≈	≈	≈	↓
SM(d18:1/24:1)	↑	≈	≈	↓
Cer(d18:0/18:0)	↑	≈	≈	≈
Cer(d18:1/18:0)	↑	≈	↑	≈
Cer(d18:1/20:0)	≈	≈	↑	≈
Cer(d18:1/22:1)	↑	≈	≈	≈
LacCer(d18:1/16:0)	↑	≈	≈	≈
LacCer(d18:1/18:0)	≈	≈	↑	≈
LacCer(d18:1/24:1)	↑	≈	≈	≈
Glc/GalCer(d18:1/16:0)	≈	≈	↑	≈
Glc/GalCer(d18:1/18:0)	≈	≈	↑	≈
PE-P(d16:0/18:2)	↓	↓	≈	≈
PC(16:0/18:2)	↓	≈	≈	↓
PC(16:0/22:6)	≈	≈	≈	↓
PC(18:0/22:6)	≈	≈	≈	↓
LPE(22:6)	≈	≈	≈	↓
CE(22:6)	≈	≈	≈	↓

QASMC study: Summary of Results – The Effects of Smoking Cessation on the Serum Lipidome

- Smoking cessation appears to result in altered serum levels of selected eicosanoids, ceramides and sphingomyelins which are associated with oxidative stress or inflammatory processes.
- The levels of the 11,12- and 14,15-DHET metabolites of AA and the related SM and Cer(d18:1/18:0) lipid molecules appear to return to never-smokers levels following cessation, and these molecules could be explored further as potential biomarkers for use in product assessment studies.
- 9-HODE and PE-P(d16:0/18:2) serum levels appear to be irreversibly affected by smoking.

QASMC Study: Why Do We Care about Nasal Scrapes ?

- COPD patients have a high prevalence of nasal symptoms (75%)
- in COPD patients, nasal inflammation mimics that of the bronchi

BMC Genomics

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Research article

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Smoking-induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium

Sriram Sridhar[†2], Frank Schembri[†1], Julie Zeskind[4], Vishal Shah[4], Adam M Gustafson[4], Katrina Steiling[1], Gang Liu[1], Yves-Martine Dumas[1], Xiaohui Zhang[1], Jerome S Brody[1], Marc E Lenburg[1,3,4] and Avrum Spira[*1,4]

Physiol Genomics 41: 1–8, 2010.
First published December 1, 2009; doi:10.1152/physiolgenomics.00167.2009.

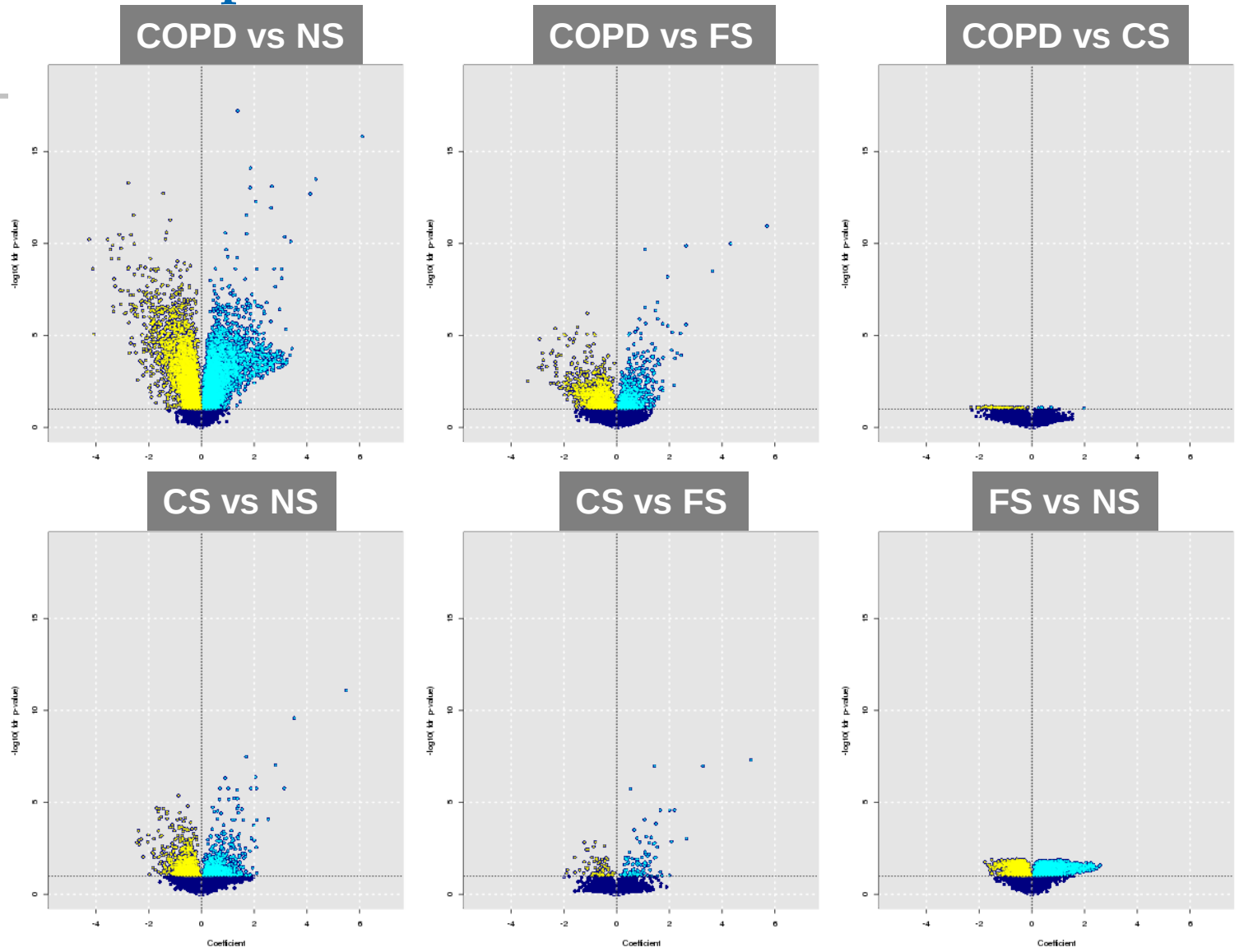
Similarities and differences between smoking-related gene expression in nasal and bronchial epithelium

Xiaoling Zhang,[1,5] Paola Sebastiani,[1,2] Gang Liu,[3,5] Frank Schembri,[3,5] Xiaohui Zhang,[3,5] Yves Martine Dumas,[3,5] Erika M. Langer,[3,5] Yuriy Alekseyev,[4] George T. O'Connor,[3] Daniel R. Brooks,[2] Marc E. Lenburg,[1,3,4,5]* and Avrum Spira[1,3,4,5]*

[1]Bioinformatics Program, Boston University; [2]Department of Biostatistics, Boston University School of Public Health; [3]The Pulmonary Center, Boston University Medical Center; [4]Department of Pathology and Laboratory Medicine, Boston University School of Medicine; and [5]Section of Computational Biomedicine, Boston University Medical Center, Boston, Massachusetts

Håkansson K, Konge L, Thomsen S et al. (2012) Sinonasal inflammation in COPD: a systematic review. European Respiratory Journal 42:1402-1411
Roberts NJ, Lloyd-Owen SJ, Rapado F et al. (2003) Relationship between chronic nasal and respiratory symptoms in patients with COPD. Respiratory medicine 97:909-914

QASMC Study: Differentially Expressed Genes in Nasal Scrapes?



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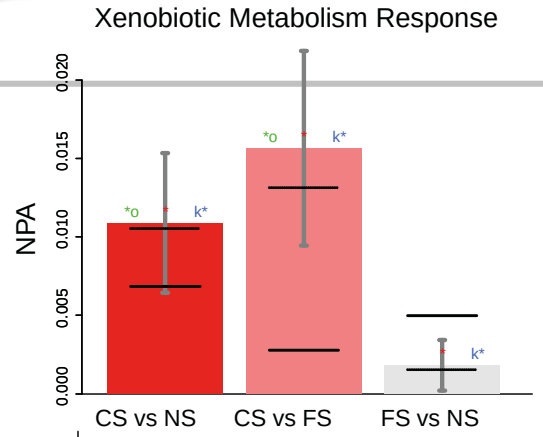
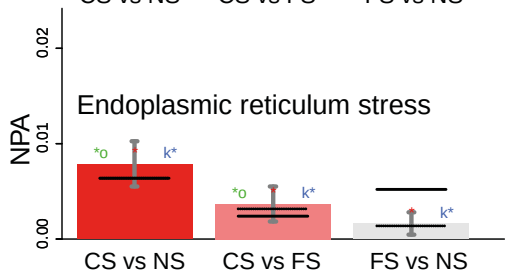
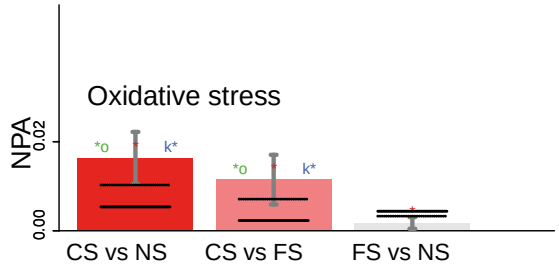
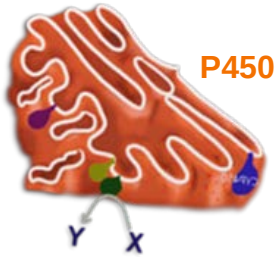
QASMC Study: The Impact of Smoking on Cellular Stress is Mostly Reversible in the Nasal Epithelium









* : P−value (w.r.t. exp replicate)< 0.05
*o: dOwnstream perm. P−value < 0.05
k*: backbone perm. P−value < 0.05
.o: 0.05 < dOwnstream perm. P−value < 0.1
k.: 0.05 < backbone perm. P−value < 0.1

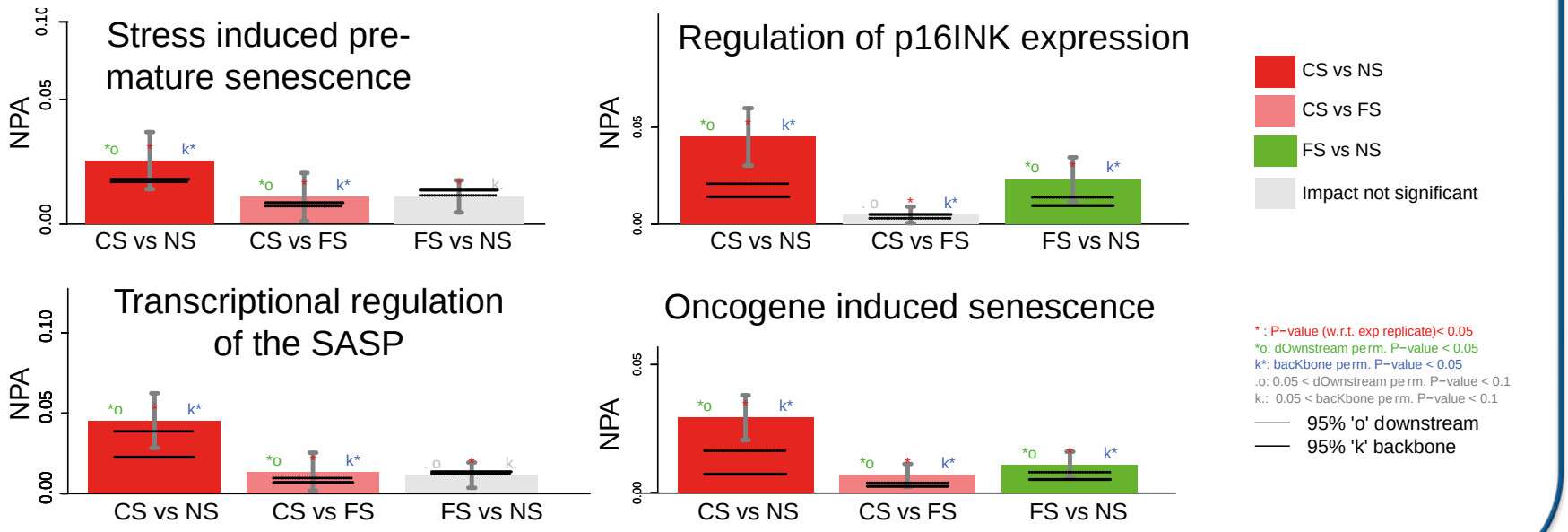
95% 'o' downstream
95% 'k' backbone

CS vs NS
CS vs FS
Impact not significant

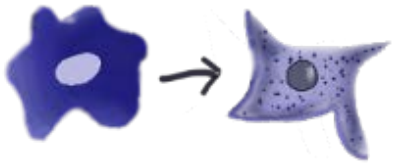
Thornton-Manning J, Dahl A (1997) Metabolic capacity of nasal tissue:: interspecies comparisons of xenobiotic-metabolizing enzymes. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 380: 43-59

Sridhar S, Schembri F, Zeskind J, Shah V, Gustafson AM, Steiling K, Liu G, Dumas Y-M, Zhang X, Brody JS (2008) Smoking-induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium. *BMC genomics 9: 259*

QASMC Study: The Impact of Smoking on Cell Senescence-related Processes is Partially Irreversible in the Nasal Epithelium



- **Cigarette smoke has an impact on senescence in lung cells, but little is known about nasal epithelium**

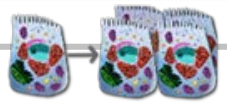


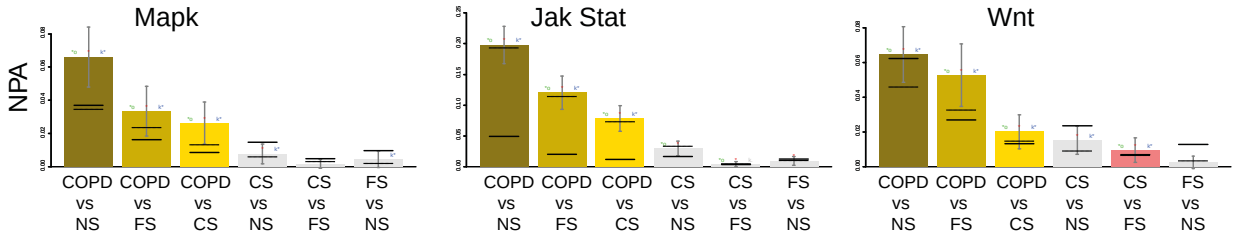
Zhou F, Onizawa S, Nagai A, Aoshiba K (2011) Epithelial cell senescence impairs repair process and exacerbates inflammation after airway injury. *Respir Res 12: 1-18*

Fujii S, Hara H, Araya J, Takasaka N, Kojima J, Ito S, Minagawa S, Yumino Y, Ishikawa T, Numata T (2012) Insufficient autophagy promotes bronchial epithelial cell senescence in chronic obstructive pulmonary disease. Oncoimmunology 1: 630

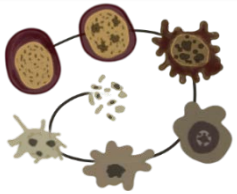
QASMC Study: COPD Specific Biology in the Nasal Epithelium

Modeled in the context of cell proliferation





Modeled in the context apoptosis



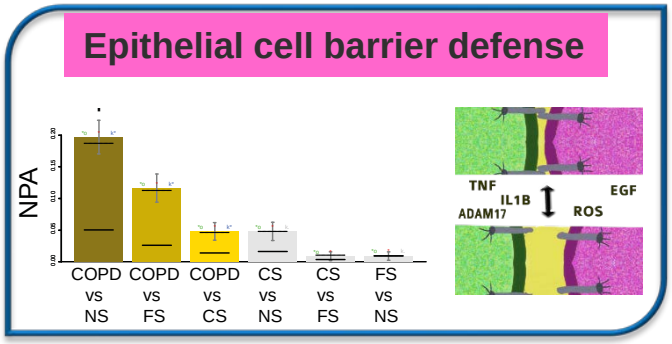
COPD vs Nonsmoker

COPD vs Former smoker

COPD vs Current smoker

Current smoker vs Former smoker

Epithelial cell barrier defense



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.o: 0.05 < dOwnstream perm. P−value < 0.1

k.: 0.05 < backbone perm. P−value < 0.1

95% 'o' downstream

95% 'k' backbone

Overall Conclusions and Next Steps

- A panel of molecular biomarkers has been identified that differentiate subjects with COPD, current and ex-smokers.
- Methods of biomarker analysis in nasal and blood samples have been optimized.
- Omics-based Systems Toxicology approach seems to be promising to link the effects observed in pre-clinical to clinical studies.
- Next steps are to validate the omics oxidative stress biomarkers and observed recovery effects in the future clinical studies including the P1 RRPs.

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