PMI SCIENCE
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Evaluation of the Tobacco Heating System 2.2 – A Candidate Modified Risk Tobacco Product

November 9th, 2016

Presented by Nikolai V. Ivanov

This research was funded by Philip Morris International

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Reduced-Risk Products ("RRPs") is the term the company uses 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 commercialization**, 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 authorization** as is the case in the US today.

Presentation Outline

1. **Introduction**
2. Eight Month ApoE$^{-/-}$ Mouse Inhalation Study for Comparative Tobacco Product Testing
3. *In vitro* Systems Toxicology for Comparative Product Testing
4. Summary of ambulatory exposure clinical ZRHM-REXA-07-JP Study Results
5. Sbv IMPROVER

PMI R&D- Background

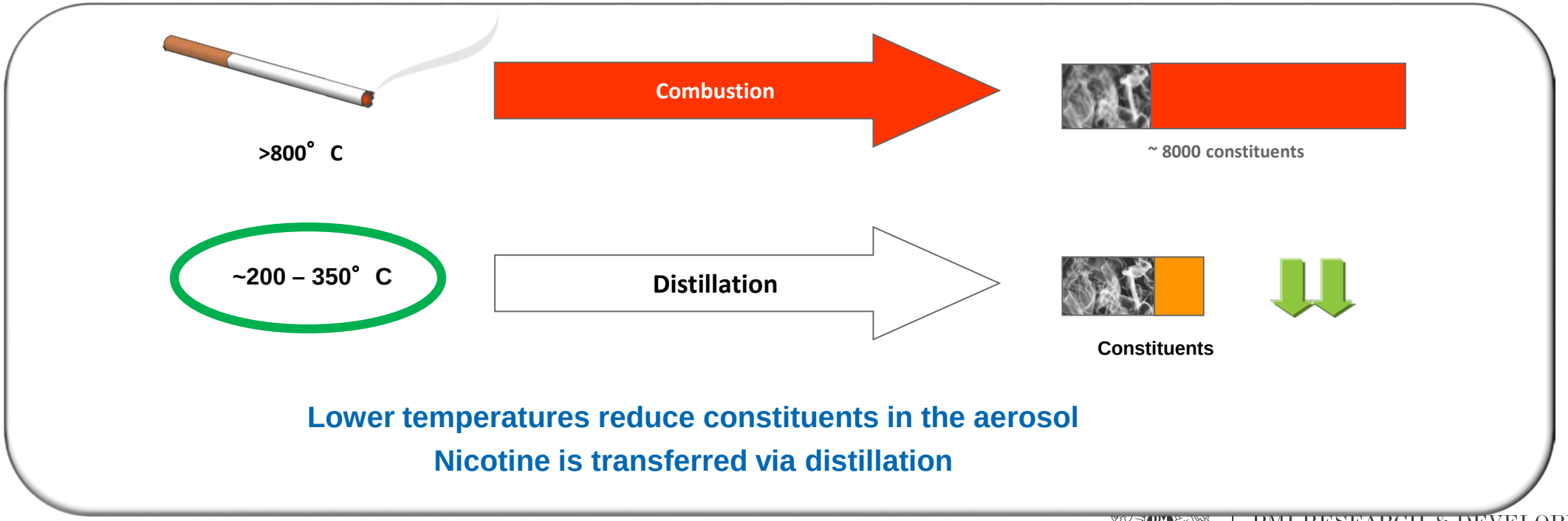
- Smoking causes serious diseases such as cardiovascular diseases, lung cancer and chronic obstructive pulmonary disease (U.S. Surgeon General, 2004)
- Smoking cessation remains the most effective approach to minimizing the risk for smoking-related diseases (Godtfredsen et al. 2008; Gepner et al., 2011)
- Providing reduced risk alternatives to adult smokers who would otherwise continue to smoke cigarettes represents the basis of the “Tobacco Harm Reduction” strategy (IOM, 2002)
- Philip Morris International (PMI) is developing novel products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes.
- To determine whether such potentially reduced-risk products (RRP) have the potential to reduce individual risk, we are conducting extensive and rigorous scientific studies comparing their biological impact with that of cigarettes.

Innovation cube, Neuchatel, Switzerland

Cigarette Smoke vs. Heat-not-Burn

Underlying Principles

- Approximately 8000 constituents identified in cigarette smoke
- Some of these constituents are categorized as harmful and potentially harmful (HPHCs)
- Many of the HPHCs are formed during combustion (burning) of the tobacco
- It is not known which HPHCs are responsible for tobacco-related diseases – selective reduction not an effective approach



Lower temperatures reduce constituents in the aerosol

Nicotine is transferred via distillation

THS2.2 – Operating Principles

Key Principles:

- Electrically heated tobacco system version 2.2 (THS2.2)
  - Tobacco plug which generates visible aerosol
  - Tobacco blends and flavor systems developed to suit lower operating temperature (< 350° C)

- Heating engine precisely controlled using built-in software
  - Heater maintains tobacco temperature in the distillation range
  - Heater also acts as a temperature sensor

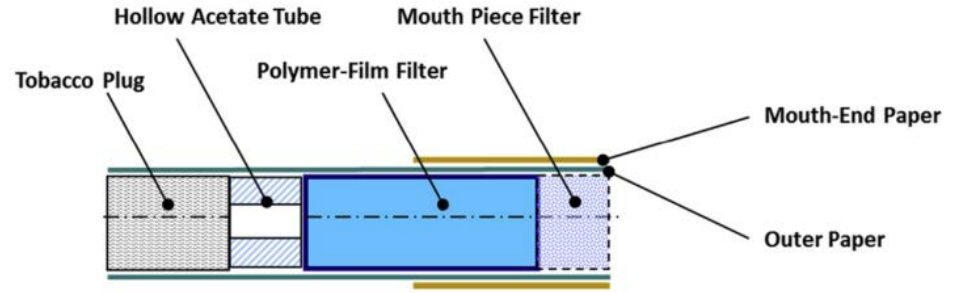


Fig. 3. Cross-sectional view of the *tobacco stick*.

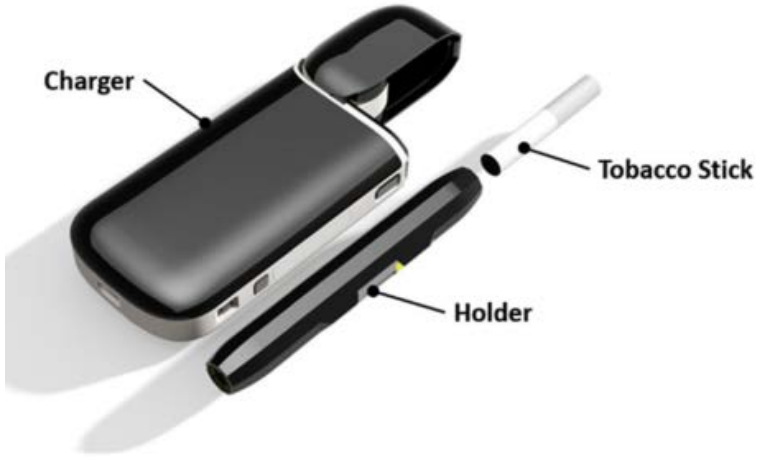
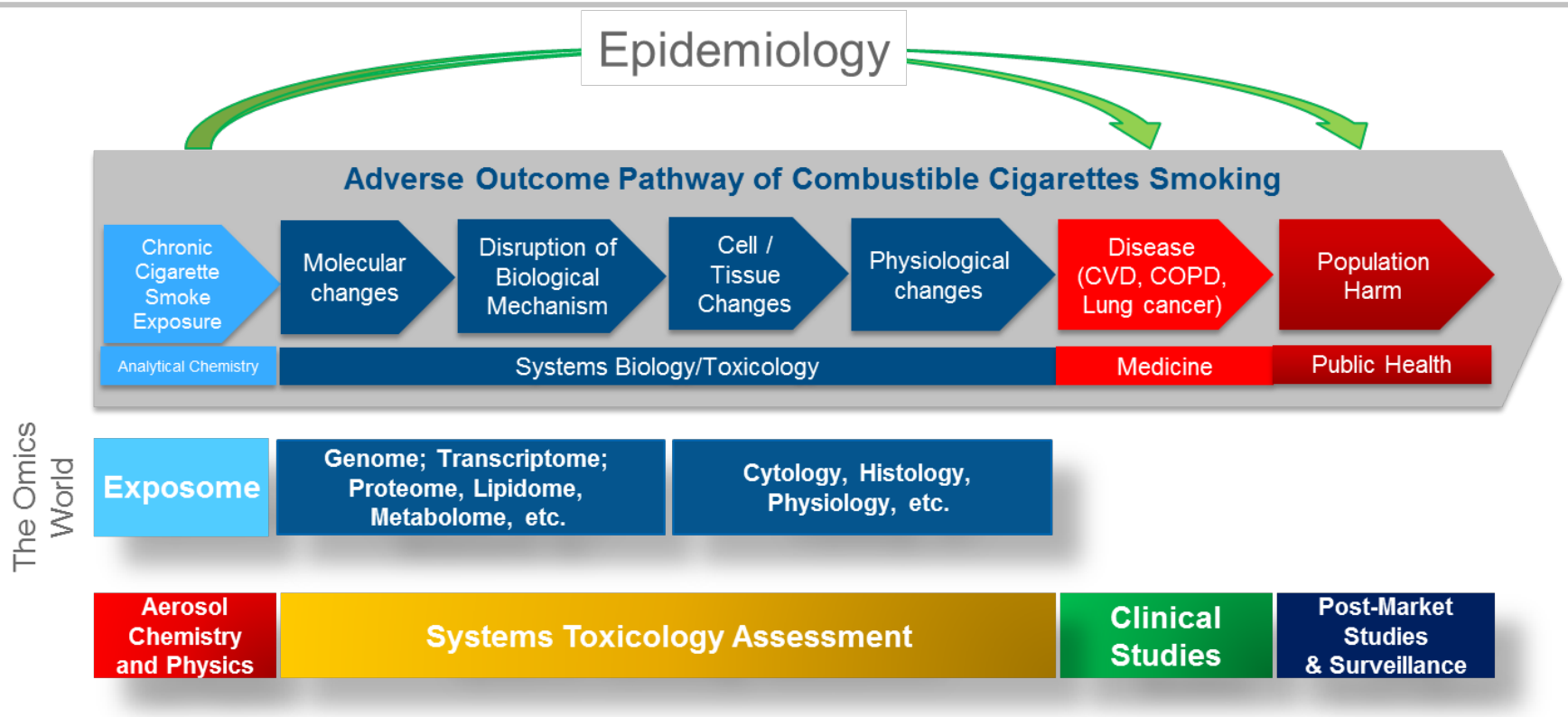


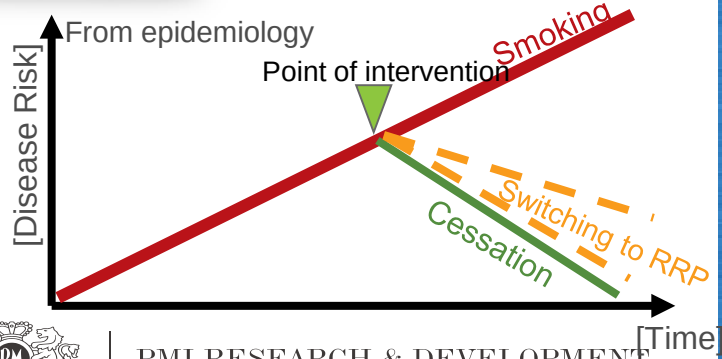
Fig. 2. The three components of the THS 2.2 product.

M.R. Smith et al. / Regulatory Toxicology and Pharmacology xxx (2016) 1–10

From Exposure to Population Harm: A Causal Chain of Events



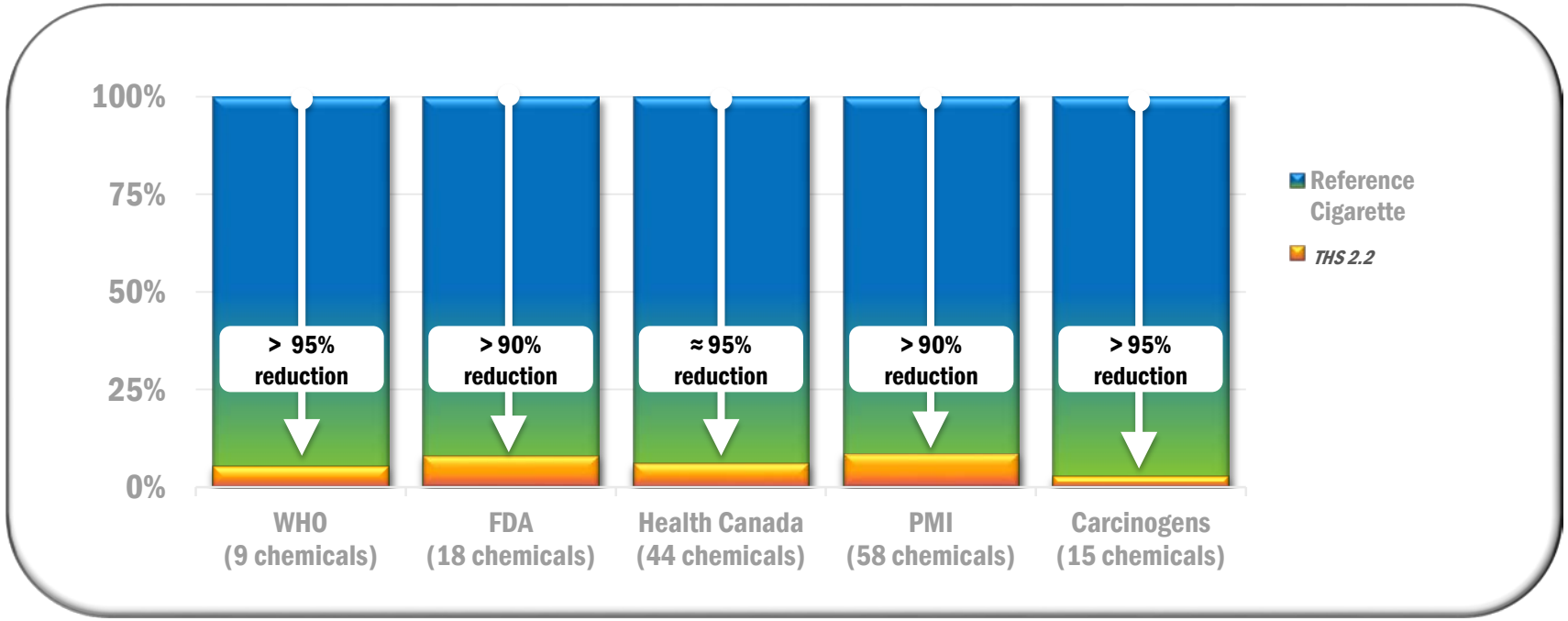
- Compare switching to RRP with continued smoking and benchmark against smoking cessation.
- Assess how close switching to RRP is to smoking cessation



Source: IOM (Institute of Medicine), 2012, Scientific Standards for Studies on Modified Risk Tobacco Products. Washington, DC: The National Academies Press

Aerosol Chemistry

Average reductions in formation of harmful or potentially harmful constituents for THS2.2 compared to levels measured in smoke from the 3R4F reference cigarette*



*Aerosol collection with Intense Health Canada's Smoking Regime (55 mL puff volume, 2 second puff duration, 30 second interval puff); Comparison on a per-stick basis Reduction calculations exclude Nicotine, Glycerin and Total Particulate Matter
The PMI 58 list includes the FDA 18,and the 15 carcinogens of the IARC Groups 1

PMI RESEARCH & DEVELOPMENT

Systems Toxicology

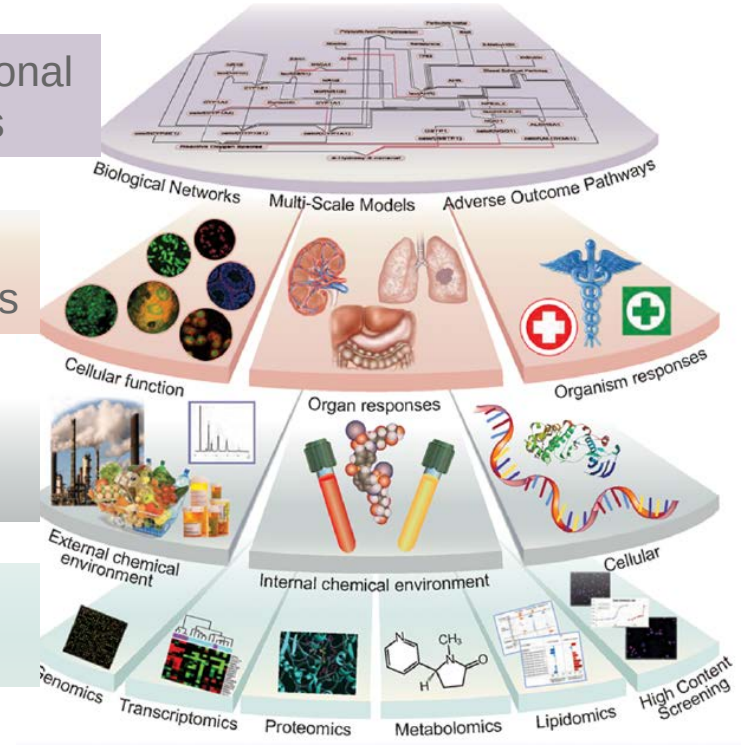
- Decoding the toxicological blueprint of active substances that interact with living systems
- Integrates classic toxicology approaches with network models and quantitative measurements of molecular and functional changes occurring across multiple levels of biological organization

Computational models

Apical measurements

Molecular measurements

Enabling technologies



Detailed mechanistic understanding of toxicology

Prediction of adverse outcomes

New paradigm for risk assessment

Environmental protection

Safe drugs

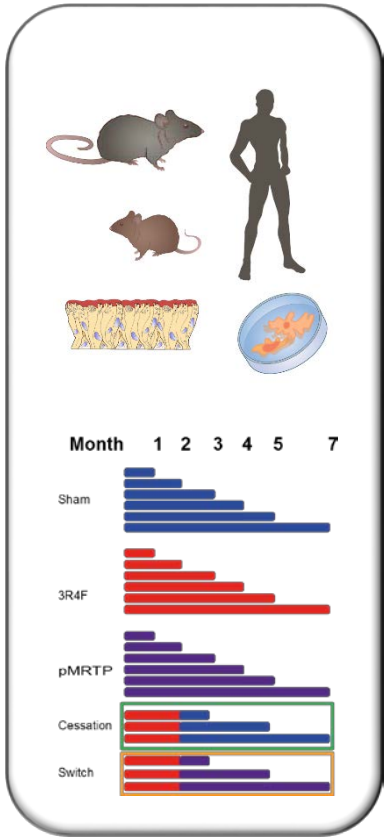
Green chemistry

Safe food

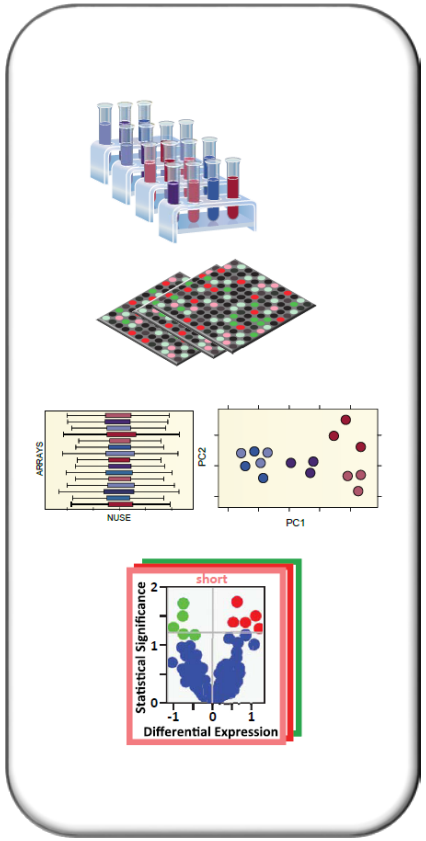
Sturla SJ, Boobis AR, Fitzgerald RE et al. (2014) Systems Toxicology: from basic research to risk assessment. Chemical research in toxicology 27:314-329

Quantitative Mechanism-Based Systems Impact Assessment

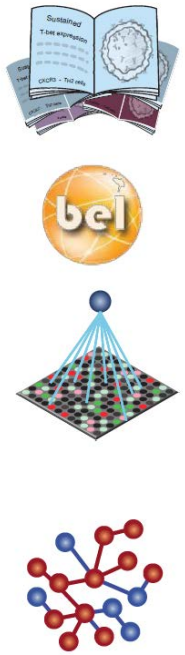
Experimental data production



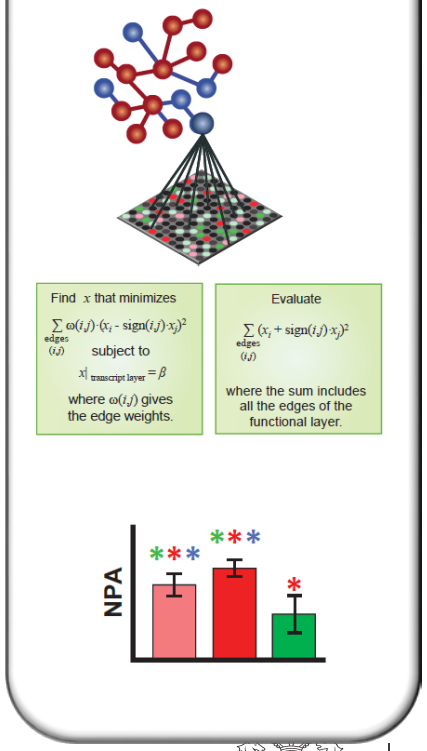
Compute systems response profiles



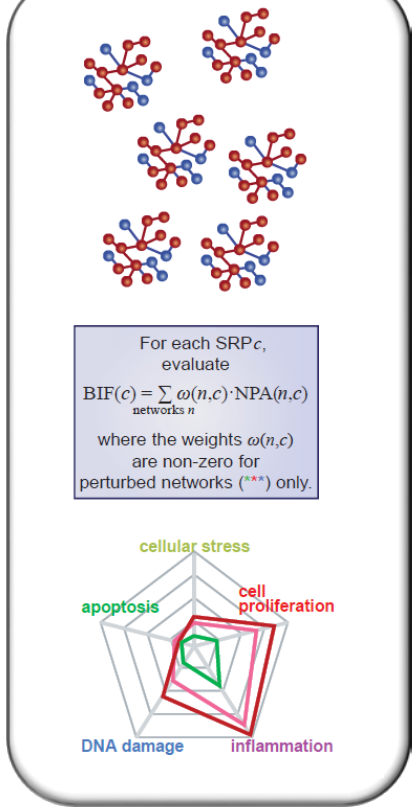
Identify perturbed biological networks



Compute network perturbation amplitudes

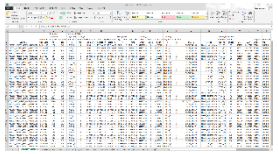


Compute product biological impact

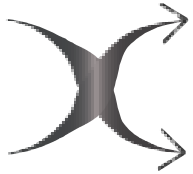


PMI RESEARCH & DEVELOPMENT

"Omics" Workflows



Biobanking and metadata preparation



Randomization

Retrieve samples in randomization order

Proteomics Workflow LC-MS (iTRAQ)

Lysis and protein precipitation

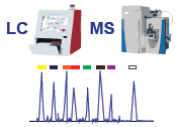
Protein concentration determination and QC

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)

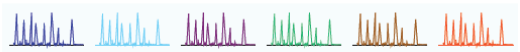
Shotgun Lipidomics (direct infusion)

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

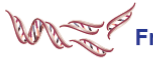
Export raw data (masses and counts of identified molecules)

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

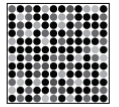
QC RNA quality and quantity

Microarray preparation

QC amplification process

Hybridization

Scanning



visual QC microarray artifacts

ELOPMENT

Presentation Outline

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Case study 1: Eight Month ApoE$^{-/-}$ Mouse Inhalation Study for Comparative Tobacco Product Testing

This research was funded by Philip Morris International

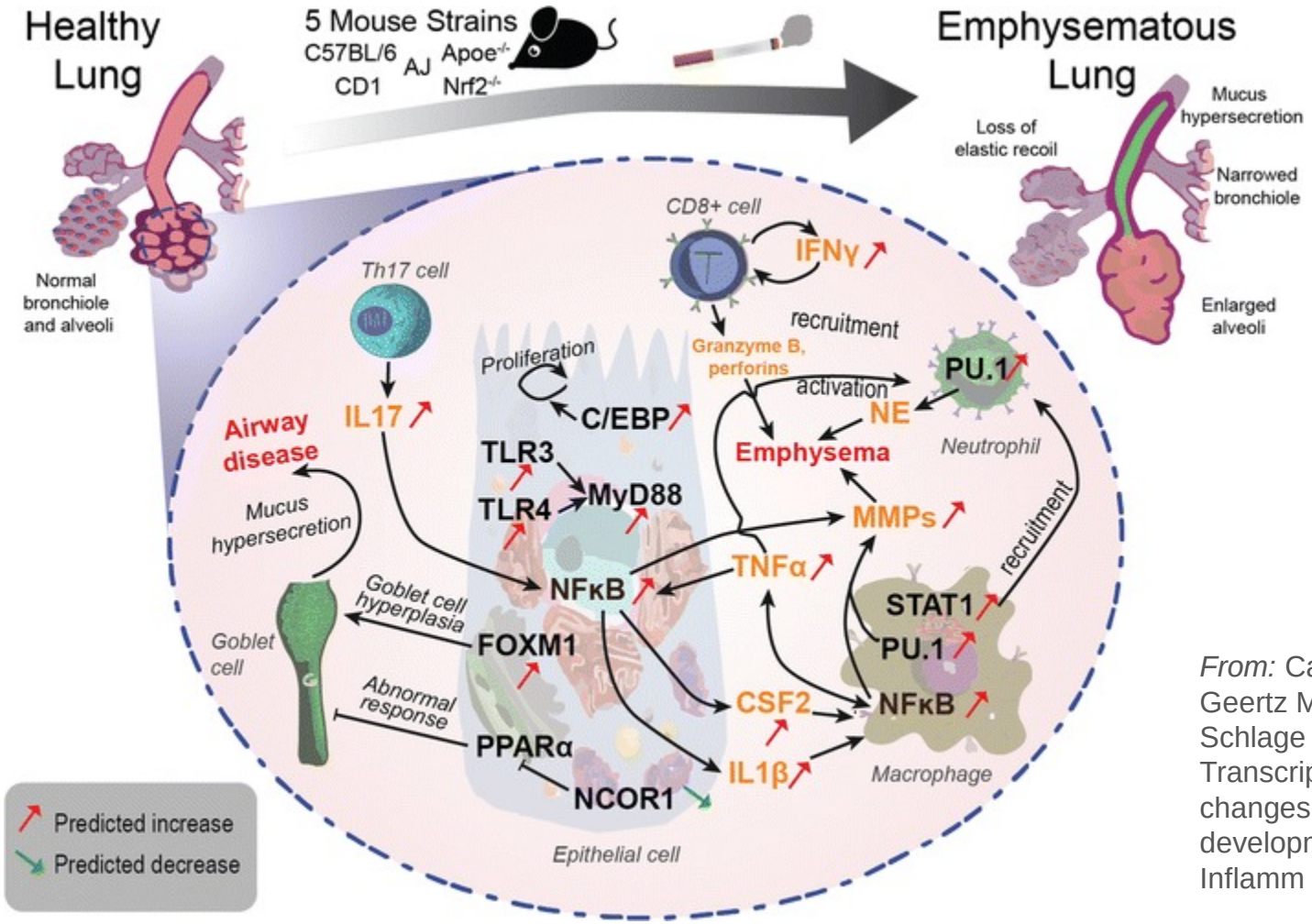
Aim and scope of the presentation

- Assessment of the effects of conventional cigarette smoke and a potential Reduced-Risk Product (pRRP), using PMI's Heat-not-Burn technology, in 2 animal models of COPD:
  - ApoE$^{-/-}$ mouse (C57Bl6 background), typically used as model for cardiovascular disease
  - A/J mouse, used as model for lung cancer
- Both animal models are responsive to cigarette mainstream smoke and develop different pathologies, among which aspects of COPD such as *lung inflammation*, changed *pulmonary function*, *emphysema**
- Other endpoints, such as general (chronic) toxicity, atherosclerosis, lung tumor development determined in these studies will not be reported here

*Lo Sasso G, Schlage WK, Boué S, Veljkovic E, Peitsch MC, Hoeng J. (2016) The Apoe(-/-) mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. J Transl Med. 2016 14(1):146.Epub. Review. **PMID 27207171**.

*Stinn W, Buettner A, Weiler H, Friedrichs B, Luetjen S, van Overveld F, Meurrens K, Janssens K, Gebel S, Stabbert R, Haussmann HJ. (2013) Lung inflammatory effects, tumorigenesis, and emphysema development in a long-term inhalation study with cigarette mainstream smoke in mice.
Toxicol Sci. 131(2):596-611. **PMID: 23104432**

Common disease mechanisms in different mouse models, relevance to human situation



- Possible interrelationships and roles for the identified common mechanisms in five mouse models of emphysema in a framework of classical human COPD mechanisms.
  - transcription factors (*black font*)
  - inflammatory mediators (*orange font*) classical pathways of human COPD pathogenesis (*black arrows*) as depicted,

From: Cabanski M, Fields B, Boue S, Boukharov N, DeLeon H, Dror N, Geertz M, Guedj E, Iskandar A, Kogel U, Merg C, Peck MJ, Poussin C, Schlage WK, Talikka M, Ivanov NV, Hoeng J, Peitsch MC. (**2015**): Transcriptional profiling and targeted proteomics reveals common molecular changes associated with cigarette smoke-induced lung emphysema development in five susceptible mouse strains. Inflamm Res.64(7):471-86. *PMID: 25962837*

Methods – Conventional cigarette smoke and aerosol from a RRP

Assessment of smoke/aerosol – Health Canada Intense smoke protocol

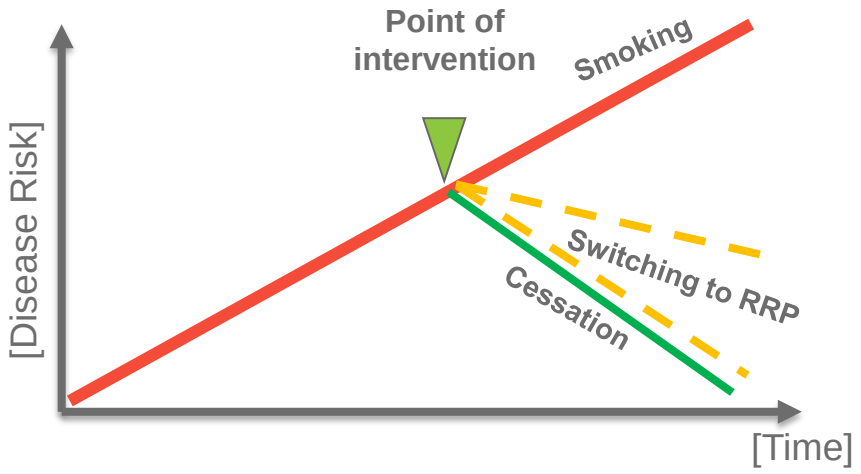
Conventional cigarettes: **Smoke** from University of Kentucky Standard Reference Cigarette 3R4F

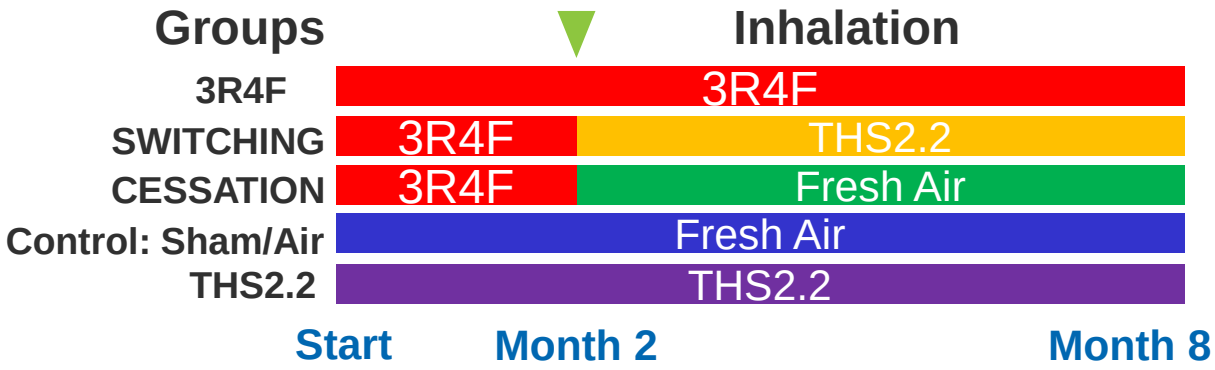
Potentially Reduced-Risk product: **Aerosol** from Heatsticks and Tobacco Heating System, THS2.2

PMI RESEARCH & DEVELOPMENT

ApoE$^{-/-}$ mouse switching study
Study design

- Comparative assessment of effects of THS2.2 and 3R4F
- Switching design upon initiation of disease:
  - to assess reversibility (switch to fresh air, i.e. cessation) and
  - To quantify how similar switching to THS2.2 is to cessation





Dissection time points: Month 1, 2, 3, 6 and 8

PMI RESEARCH & DEVELOPMENT

ApoE$^{-/-}$ mouse switching study
Methods - Exposure regime

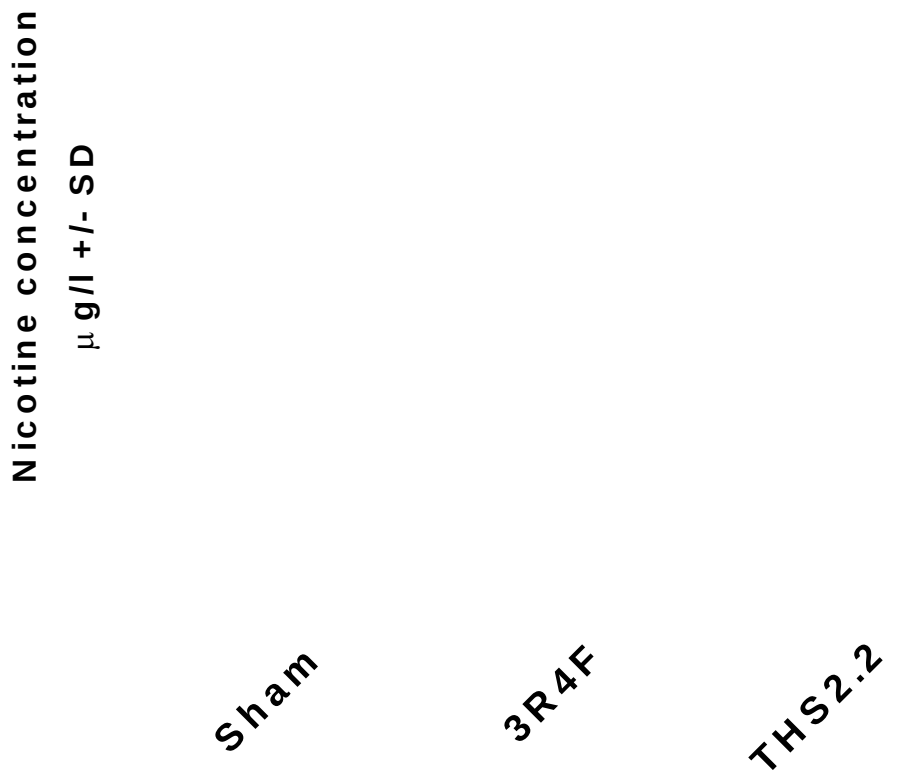
1 hour	30 min	1 hour	1 hour	1 hour

Fresh air

THS2.2 or 3R4F

- Animals were exposed 3 hours per day (3 x 1 hour interrupted exposure periods), 5 days per week
- Nicotine was measured during every exposure period (3 samples per chamber per day)
- Aerosol delivery (nicotine) was within +/- 10% of the targeted **29.9 µg/l** nicotine concentration

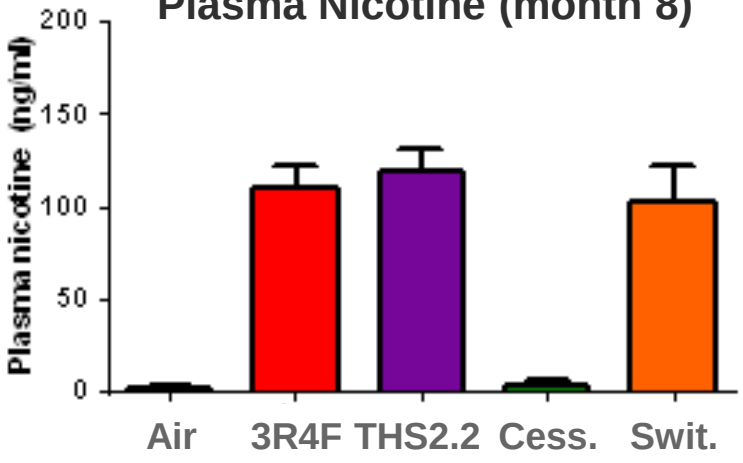
Nicotine concentration in exposure chamber (study average)



*29.9 µg/l nic corresponds to 6.5 mg/kg, daily dose- or the nicotine amount from approx. 32 cig/day for a 60 kg human, based on body surface comparison, Guidance document Heq dose, FDA

ApoE$^{-/-}$ mouse switching study
Aerosol uptake (biomarkers of exposure)

Plasma Nicotine (month 8)

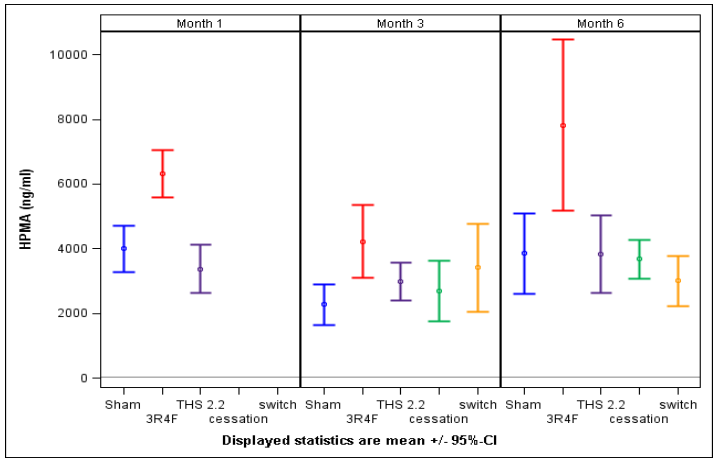


Means ± SEM

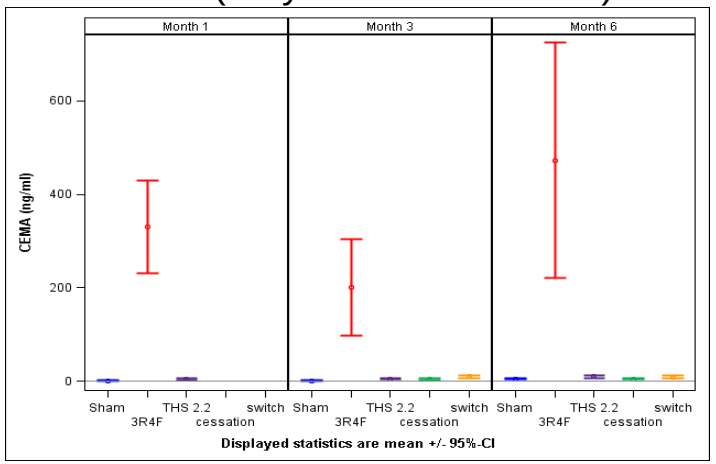
Groups
- 3R4F
- Cessation
- Switching
- THS2.2
- Sham / Air

Urinary Metabolites (months 3, 6, 8)

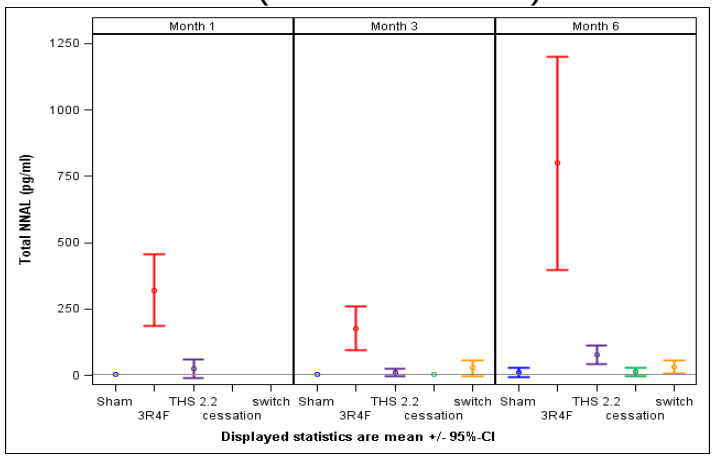
HPMA (Acrolein metabolite)



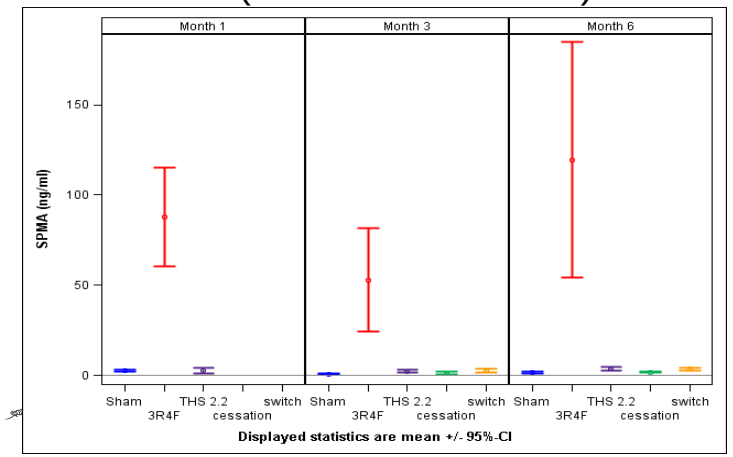
CEMA (Acrylonitrile metabolite)



NNAL (NNK metabolite)



SPMA (Benzene metabolite)

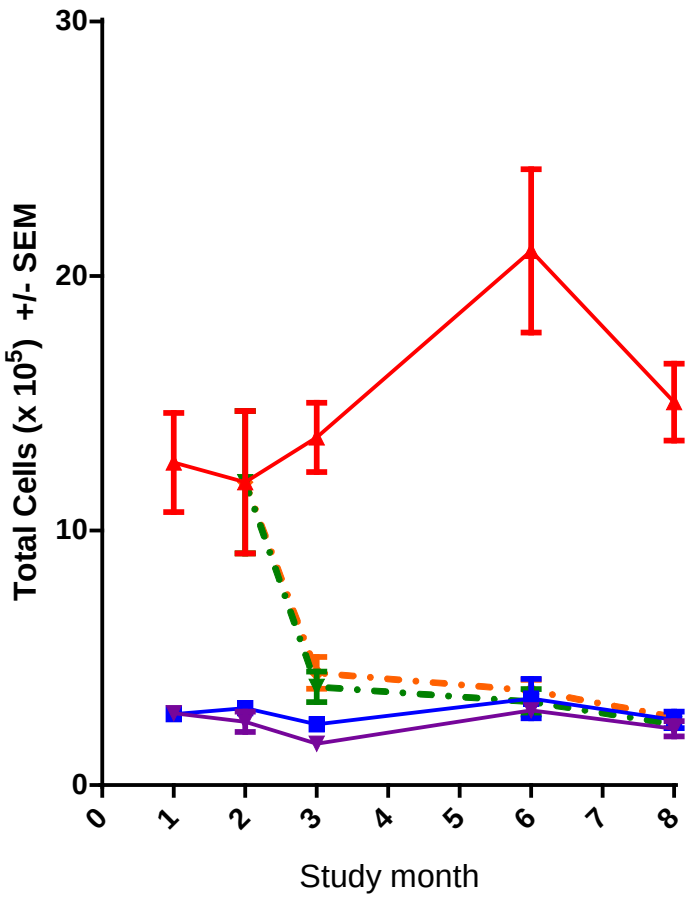


Means ± SEM

ApoE$^{-/-}$ mouse switching study
Result summary: Disease mechanisms - Lung inflammation

Free lung cells in Broncho-alveolar lavage fluid (BALF)

Total cells absolute



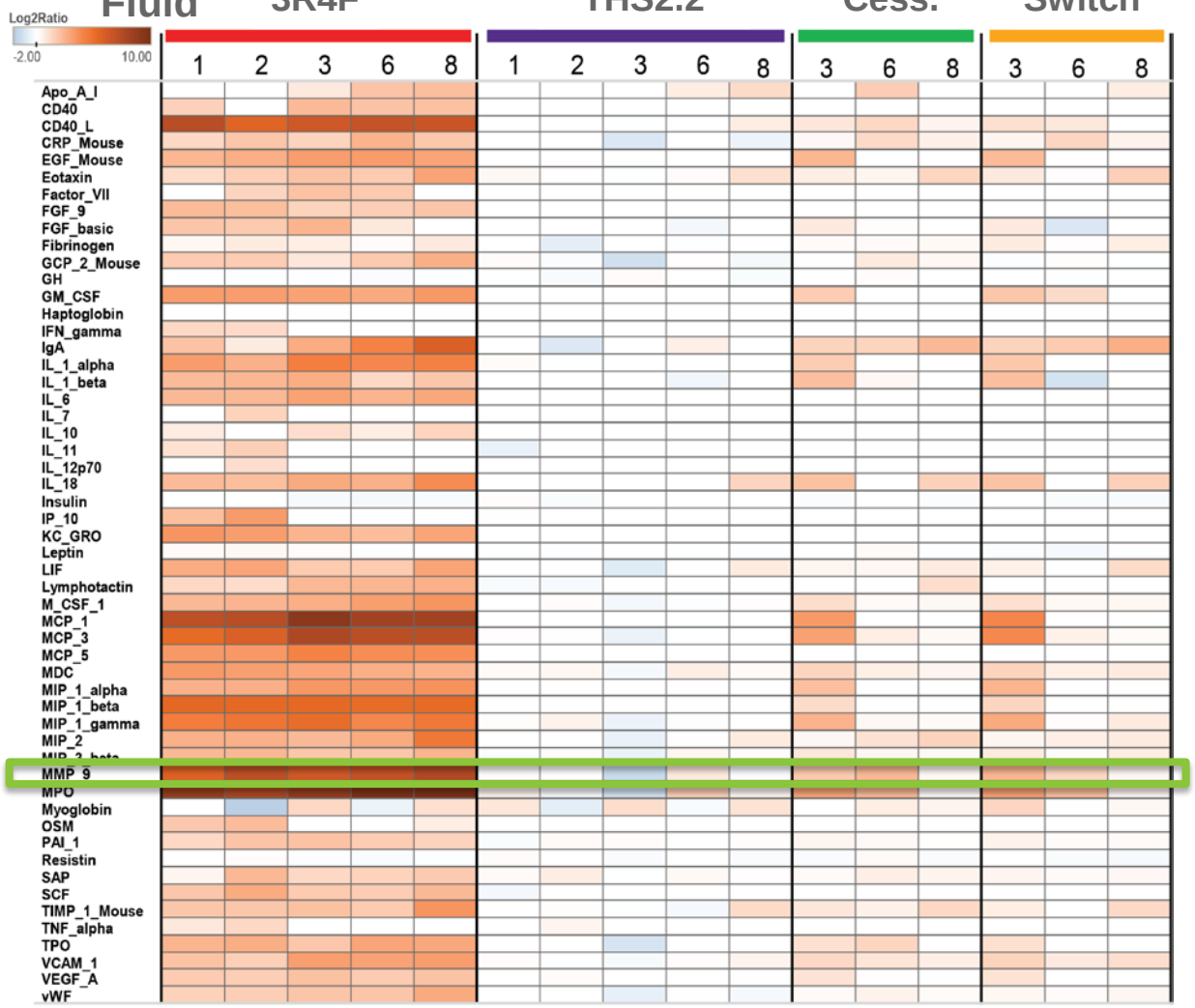
Differential counts

Groups
- 3R4F
- Cessation
- Switching
- THS2.2
- Sham / Air

Study month

ApoE$^{-/-}$ mouse switching study
Result summary: Disease mechanisms - Lung inflammation

Multiple analyte profiling in Broncho-alveolar Lavage Fluid



MMP activity (mU/ml) +/- SD

Groups

- 3R4F
- Cessation
- Switching
- THS2.2
- Sham / Air

PMI RESEARCH & DEVELOPMENT

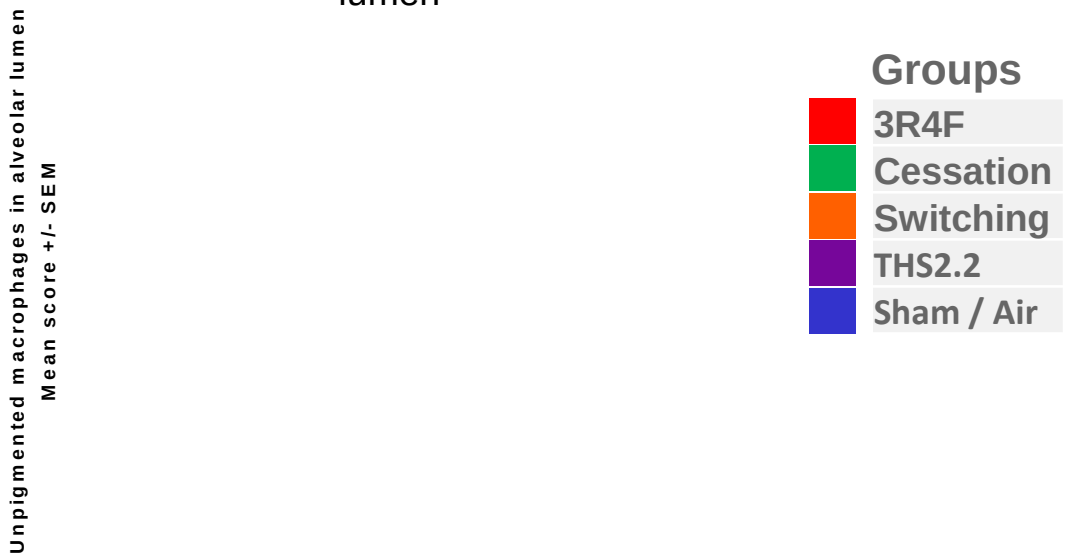
ApoE$^{-/-}$ mouse switching study
Result summary: Disease endpoints- Lung function and lung volume

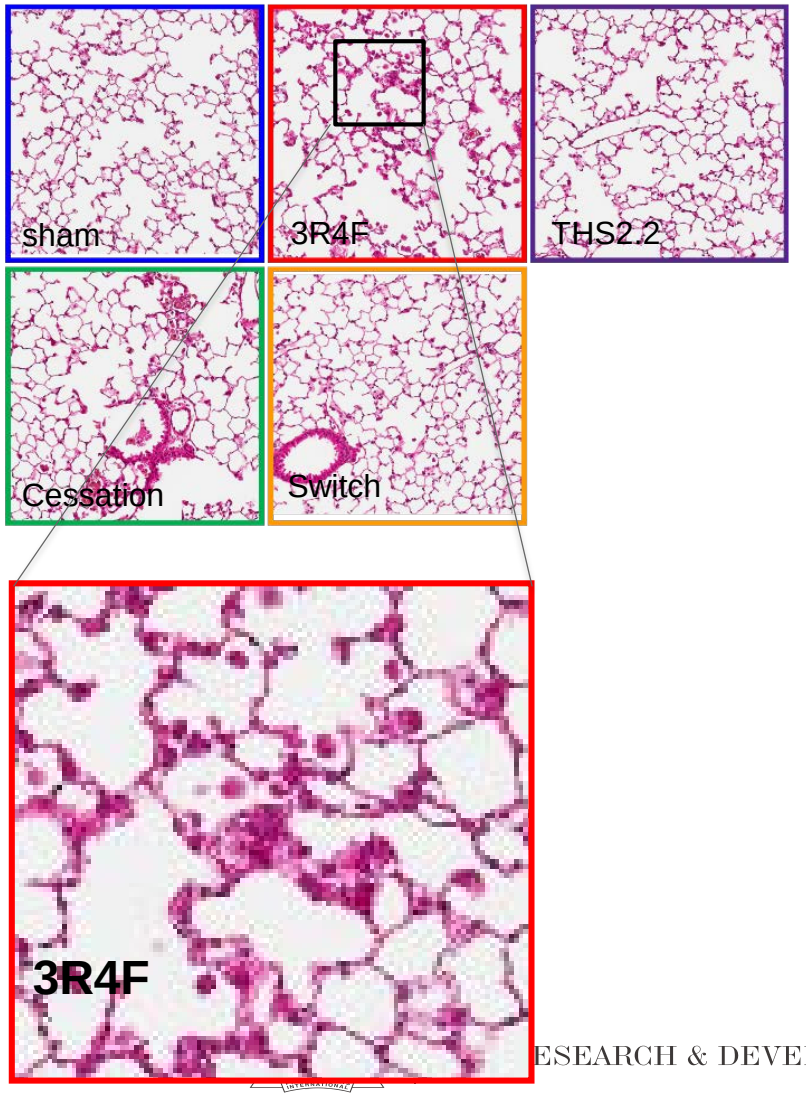
Lung function: Pressure Volume Loops (PVsP) (FlexiVent (Scireq))

Means ± SEM

ApoE$^{-/-}$ mouse switching study
Result summary: Histopathology of the lung – Pulmonary inflammation

Results, Lung Inflammation
unpigmented macrophages in the alveolar lumen





*: Statistically significant compared to sham
*: Statistically significant compared to 3R4F:CONT at month 2

- Decrease in mean scores after switching to fresh air or THS2.2 (statistically significant from month 6)
- No statistically significant difference between Cessation group and THS2.2-Switch group at month 3

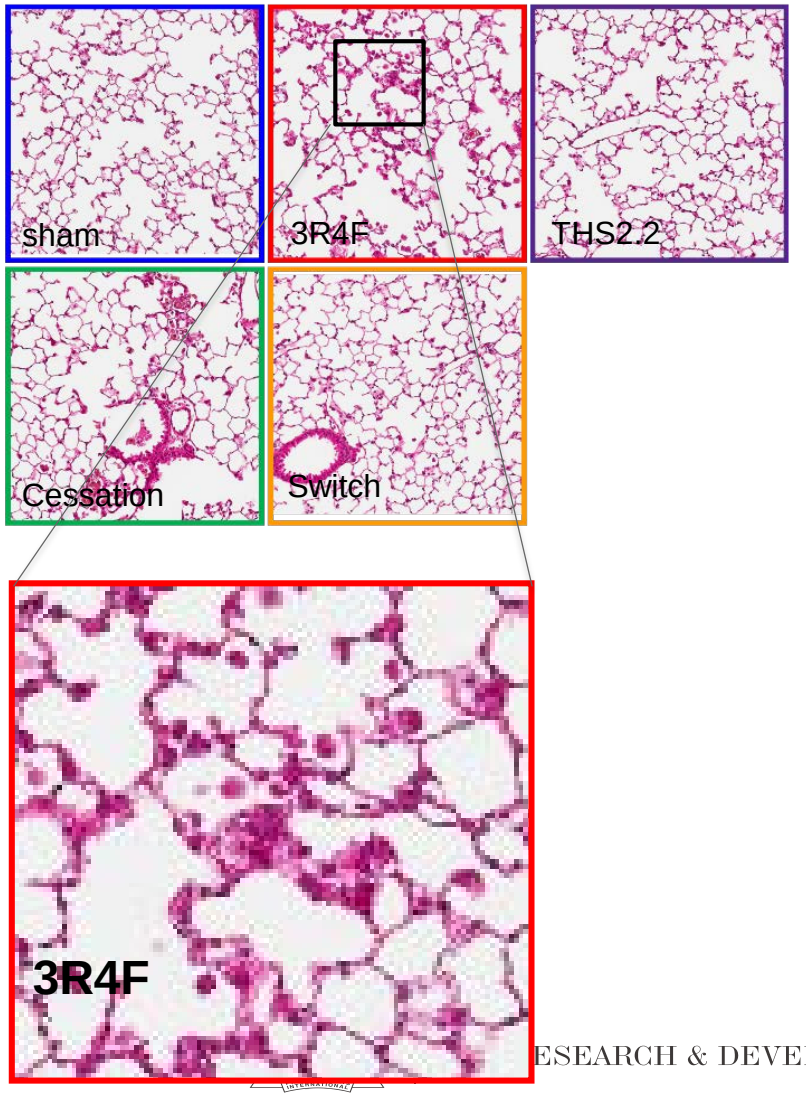
ESEARCH & DEVELOPMENT

Switching Study in an Animal Model of Disease
Result summary: Tissue changes – Histopathology

Histopathological Assessment emphysema

Alveolar emphysema +/- SEM

Groups
- 3R4F
- Cessation
- Switching
- THS2.2
- Sham / Air



*: Statistically significant compared to sham
*: Statistically significant compared to 3R4F:CONT at month 2

ESEARCH & DEVELOPMENT

ApoE$^{-/-}$ mouse switching study
Result Summary: Lung tissue changes – Morphometry

Bronchiolar attachments
- Fewer Bronchiolar attachments in 3R4F-exposed group

Mean chord length (MCL)
- Mean linear intercept length
- Increased MCL in 3R4F-exposed group

Destructive index (DI)
- Index of parenchymal destruction
- Increased DI in 3R4F-exposed group

PMI RESEARCH & DEVELOPMENT

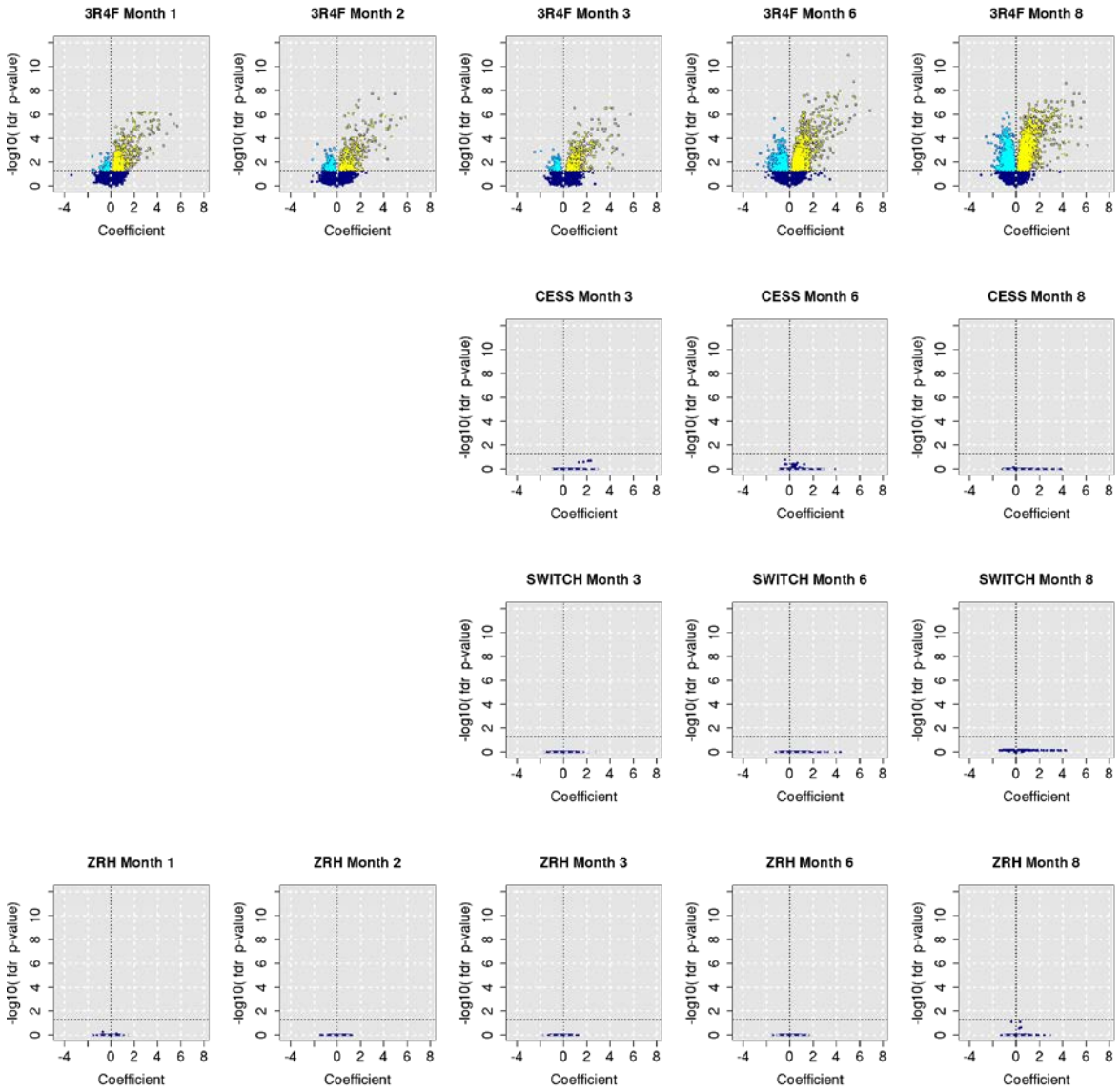
ApoE$^{-/-}$ mouse switching study
Result Summary: Systems response profile: differential gene expression - Lung

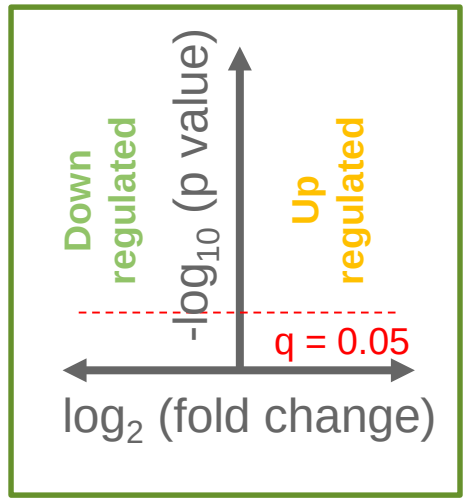
3R4F

Cessation

Switch

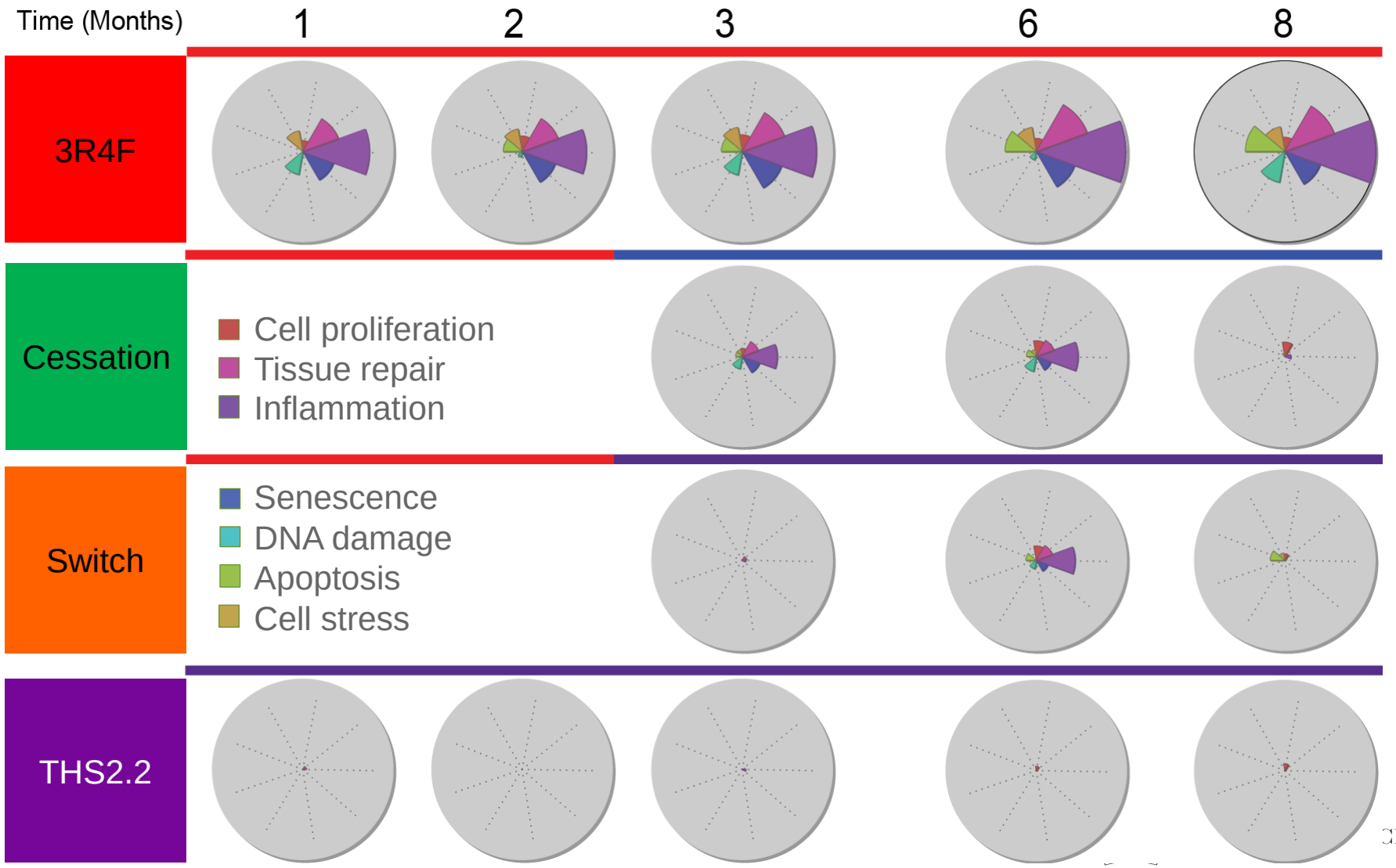
THS2.2





ESEARCH & DEVELOPMENT

ApoE$^{-/-}$ mouse switching study
Result Summary: Disease mechanisms - Network perturbations - Lung



CH & DEVELOPMENT

Switching Study in an Animal Model of Disease
Summary and Conclusions

- The ApoE$^{-/-}$ mouse model is suitable for studying smoke-related aspects of COPD
- Continuous exposure to smoke from 3R4F causes lung inflammation, lung function and emphysematous changes as of one month of treatment
- Continuous exposure to aerosol from THS2.2 for up to 8 months does not increase inflammation and emphysema in comparison to Sham group
- Switching from cigarette smoke exposure after 2 months to fresh air (Sham) exposure reverses the onset of disease as measured in apical, functional, and molecular endpoints
- Switching from cigarette smoke exposure to THS2.2 aerosol exposure reverses the onset of disease in a similar manner as cessation

Phillips B, Veljkovic E, Boué S, Schlage WK, Vuillaume G, Martin F, Titz B, Leroy P, Buettner A, Elamin A, Oviedo A, Cabanski M, De León H, Guedj E, Schneider T, Talikka M, Ivanov NV, Vanscheeuwijck P, Peitsch MC, Hoeng J. (2016). 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 CigarettesToxicol Sci. 149(2):411-32. **PMID 26609137.**

Titz, B., Boue, S., Phillips, B., Talikka, M., Vihervaara, T., Schneider, T., Nury, C., Elamin, A., Guedj, E., Peck, M.J., Schlage WK, Cabanski M, Leroy P, Vuillaume G, Martin F, Ivanov NV, Veljkovic E, Ekroos K, Laaksonen R, Vanscheeuwijck P, Peitsch MC, Hoeng J.(2016). 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. Toxicol Sci *149*, 441-457. **PMID 26582801.**

Lo Sasso, G., Titz, B., Nury, C., Boue, S., Phillips, B., Belcastro, V., Schneider, T., Dijon, S., Baumer, K., Peric, D, Dulize R, Elamin A, Guedj E, Buettner A, Leroy P, Kleinhans S, Vuillaume G, Veljkovic E, Ivanov NV, Martin F, Vanscheeuwijck P, Peitsch MC, Hoeng J. (2016). Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver in Apoe−/− mice – a systems toxicology analysis. Inhal Toxicol. 28(5): 226-4. **PMID 27027324.**

Lo Sasso G, Schlage WK, Boué S, Veljkovic E, Peitsch MC, Hoeng J. (2016) The Apoe(-/-) mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. J Transl Med. 2016 14(1):146.Epub. Review. **PMID 27207171**.

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Ongoing study: A/J Mouse Study Design (OECD TG 453 – Chronic toxicity & Carcinogenicity Study)

This research was funded by Philip Morris International

Study Objective

- Objective: To assess the impact of lifetime exposure to THS aerosol, compared with 3R4F cigarette smoke, on development of emphysema and on lung tumor incidence and multiplicity in a 18-month exposure study in A/J mice.

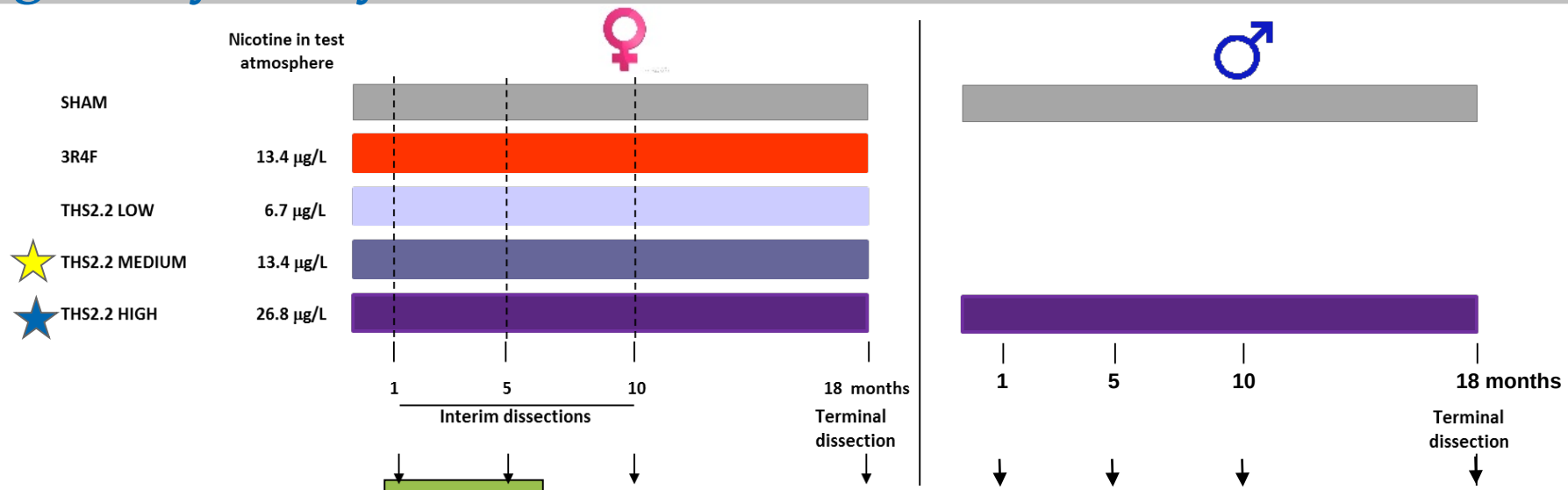
- Rationale for the use of A/J mouse strain:

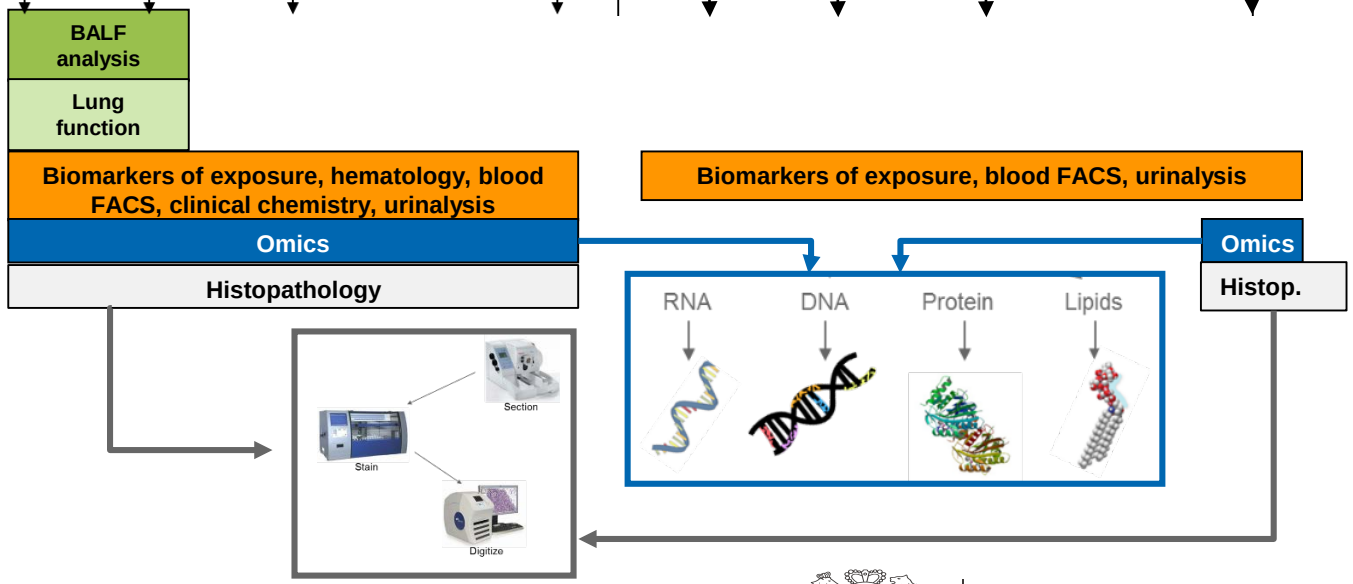
  The A/J mouse is highly susceptible to lung tumor induction. Several studies including ours showed aerosol concentration dependent increased lung tumors following exposure to mainstream cigarette smoke (MS) in A/J mice. (Coggins, 1998; Stinn, 2005; Stinn, 2010)

  Transcriptomics analysis demonstrated differences between lung tumors that developed from MS-exposed versus spontaneously arising tumors (Luettich, 2014)

  A/J mice also develop inflammatory and emphysematous changes following chronic exposure to MS (Stinn et al., 2012, 2013)

A/J Mouse Study Design (OECD TG 453 – Chronic toxicity & Carcinogenicity Study)





★ Equivalent to the 300 µg TPM/l concentration in the previous studies (Stinn et al., 2012, 2013)

★ 26.8 µg/l nic (high dose) corresponds to 11.6 mg/kg, daily dose- or the nicotine amount from approx. 56 cig/day for a 60 kg human, based on body surface comparison, Guidance document Heq dose, FDA

PMI RESEARCH & DEVELOPMENT

Presentation Outline

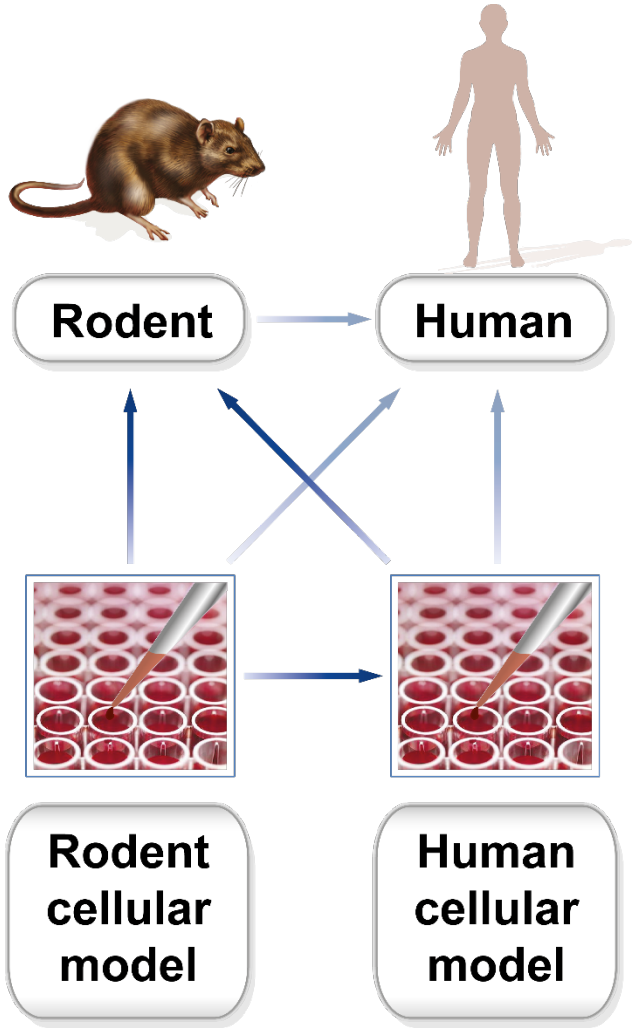
1. Introduction
2. Eight Month ApoE$^{-/-}$ Mouse Inhalation Study for Comparative Tobacco Product Testing
3. ***In vitro* Systems Toxicology for Comparative Product Testing**
4. Summary of ambulatory exposure clinical ZRHM-REXA-07-JP Study Results
5. Sbv IMPROVER

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Case study 2: *In vitro* Systems Toxicology for Comparative Product Testing

This research was funded by Philip Morris International

Establishment of Reliable in vitro Systems Supports the Principles of 3Rs



Translation between species and experimental systems

PMI RESEARCH & DEVELOPMENT

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

Background - Replacing, Reducing and Refining

2009: European Commission published a report on «Alternative Testing Strategies for «Replacing, Reducing, and Refining» («3R») the 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, muco-ciliary clearance,..) and allowing whole cigarette smoke exposure at the air-liquid interface.**

"The Food, Drug, and Cosmetic Act directs the FDA to consult with the Institute of Medicine (IOM) on the design and conduct of studies for the assessment of MRTPs. In its 2011 report, *Scientific Standards for Studies on Modified Risk Tobacco Products*, the IOM identified, as a standard step in this assessment, *in vitro* tests for cytotoxicity, genotoxicity, proliferation, apoptosis, oxidative stress, inflammation, mucus production, and endothelial cell activation."

PETA issued a letter in March 2014 (include reference at the bottom to the link) whereby PETA stated the in-vitro field has progressed significantly with many varied technological advances and the in vitro testing of tobacco products is no longer "limited to a small number of cytotoxicity and genotoxicity assays"

PCRM PHYSICIANS COMMITTEE FOR RESPONSIBLE MEDICINE
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WASHINGTON, DC 20016
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PCRM@PCRM.ORG WWW.PCRM.ORG

March 26, 2014

Caryn Cohen, M.S.
Office of Science
Center for Tobacco Products
Food and Drug Administration
9200 Corporate Blvd.
Rockville, MD 20850

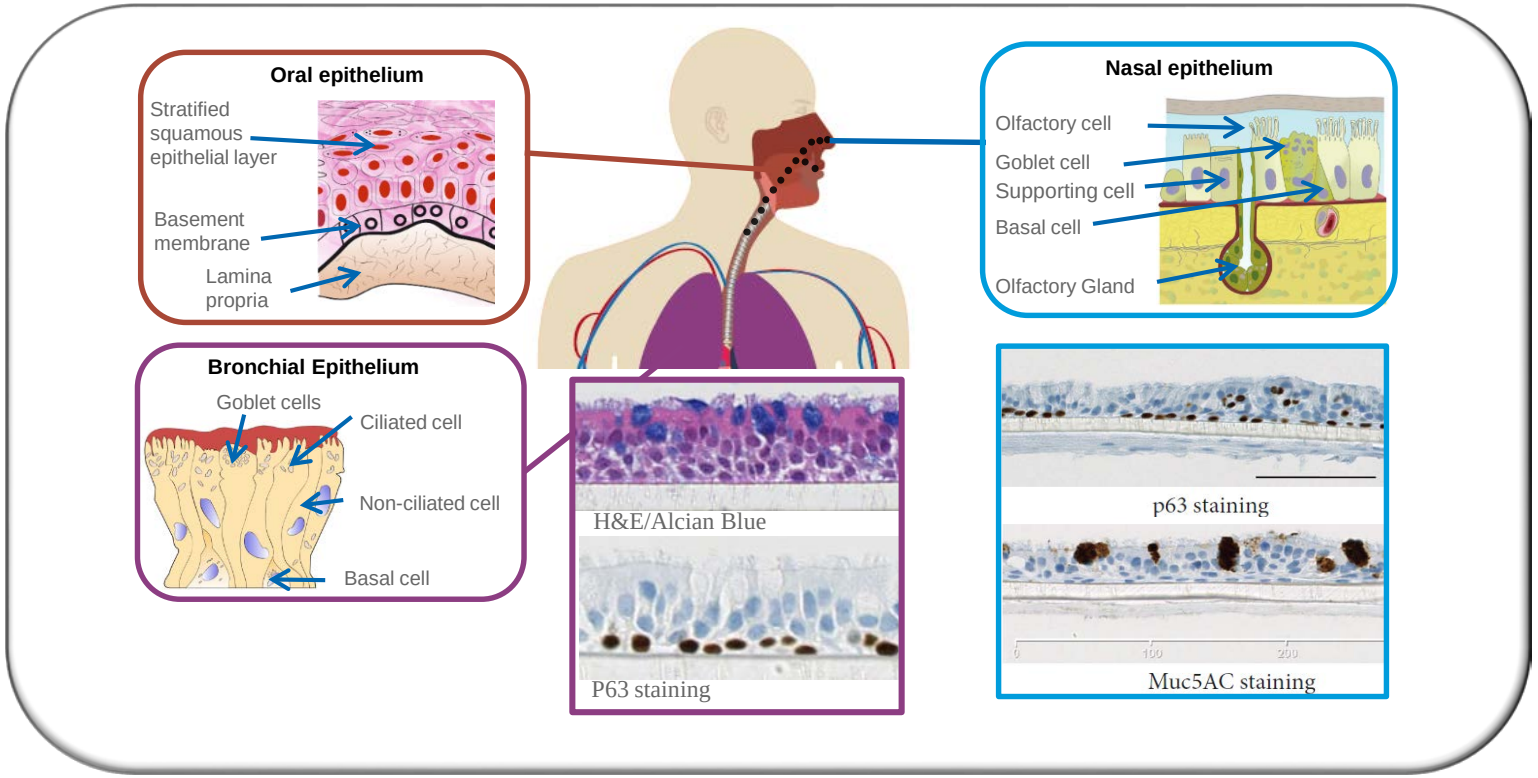
PETA
PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

HEADQUARTERS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

Belgium, Estonia, Germany, UK and Slovakia have banned animal testing for tobacco testing

PMI RESEARCH & DEVELOPMENT

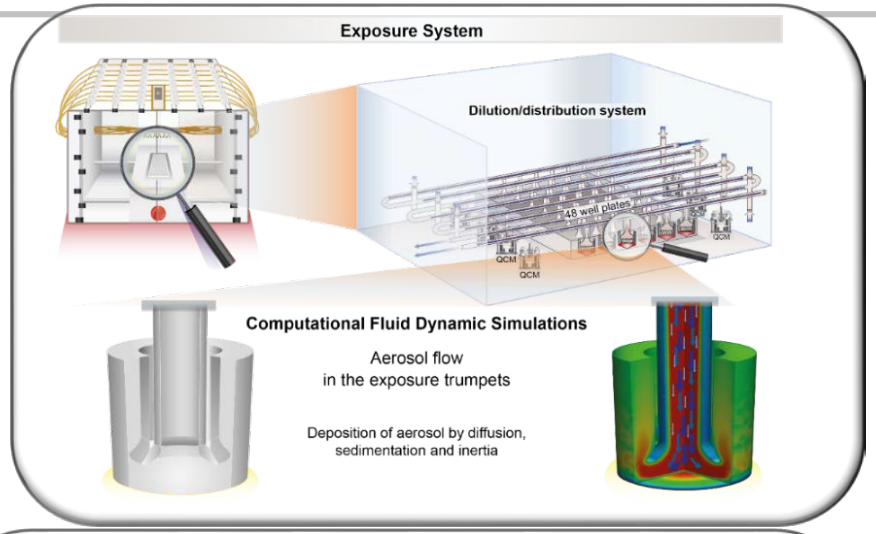
Organotypic Bronchial, Nasal, and Oral Tissue Cultures Resemble *in vivo* Respiratory Epithelium

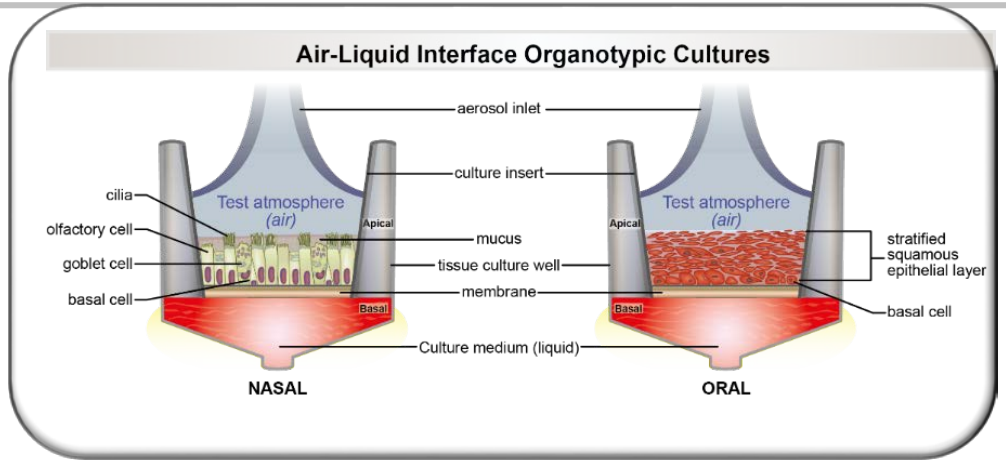


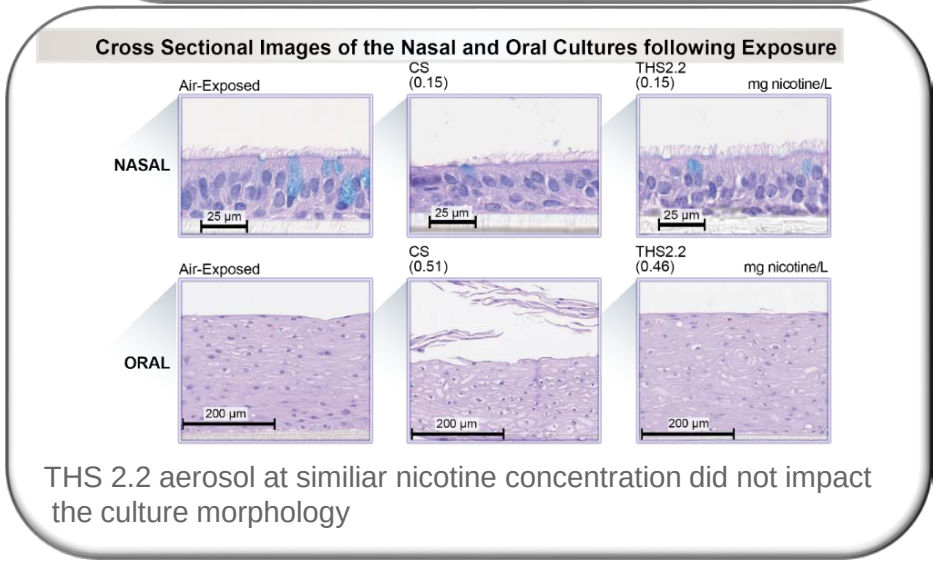
- Sampling the bronchial epithelium to identify potential biomarkers of exposure response and disease has yielded significant insights.
- Many of the smoking-related changes in the bronchial epithelium are also present in the nasal and buccal epithelium.

Sridhar et al: Smoking-induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium. BMC genomics 9: 259 (2008)
Iskandar et al: Systems approaches evaluating the perturbation of xenobiotic metabolism in response to cigarette smoke exposure in nasal and bronchial tissues. BioMed research international. 2013;2013.

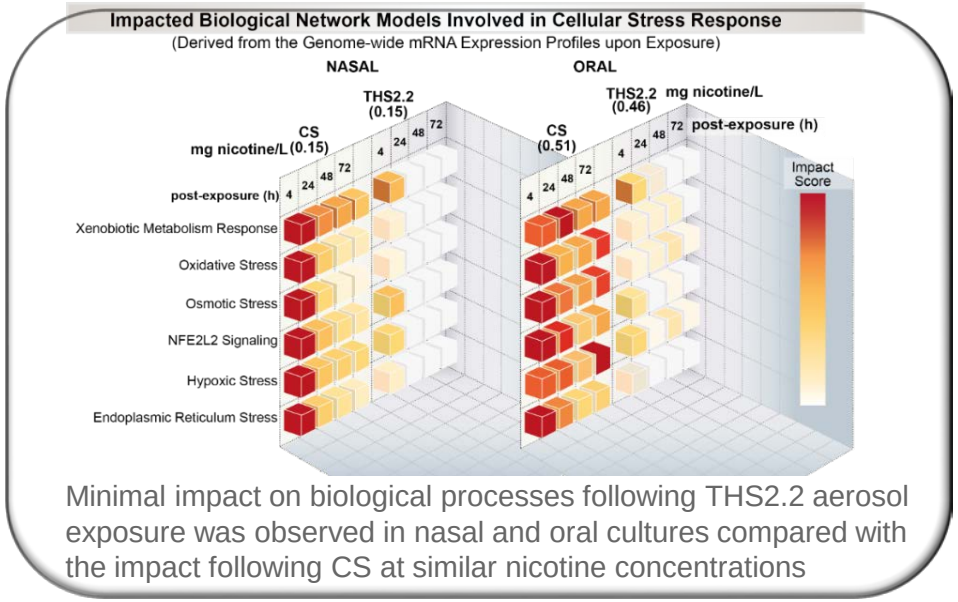
Reduced Biological Impact of THS2.2 Aerosol Exposure was Observed in Human Nasal and Oral Cultures Compared with CS at Comparable Nicotine Concentrations







THS 2.2 aerosol at similiar nicotine concentration did not impact the culture morphology

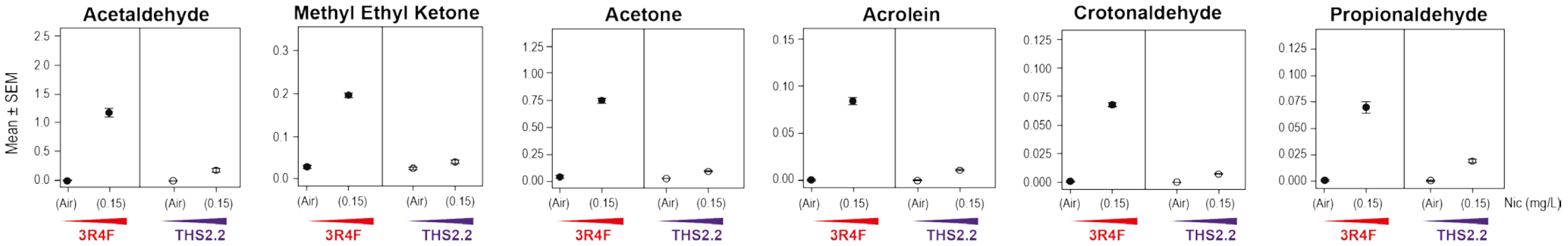


Minimal impact on biological processes following THS2.2 aerosol exposure was observed in nasal and oral cultures compared with the impact following CS at similar nicotine concentrations

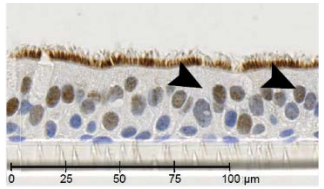
Zanetti et al: Systems toxicology assessment of the biological impact of a candidate Modified Risk Tobacco Product on human organotypic oral epithelial cultures. Chemical Research in Toxicology 2016, 29 (8), 1252-1269.

Reduced Biological Effects of THS2.2 Aerosol Exposure in Human Nasal Cultures as Compared to CS at comparable nicotine concentration

Concentrations of Representative Carbonyls (µg/mL)

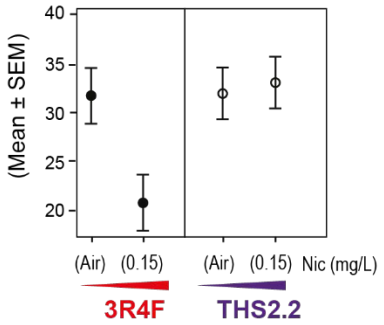


FoxJ1-Marker of Ciliated Cells

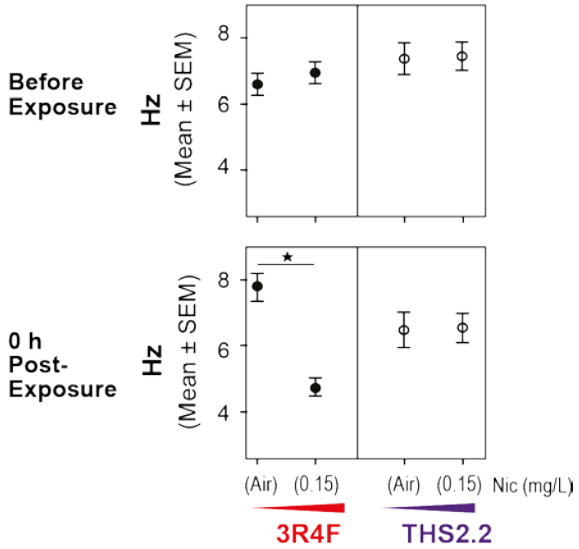


Proportion of FoxJ1-Stained Cells

(72 h Post-Exposure)

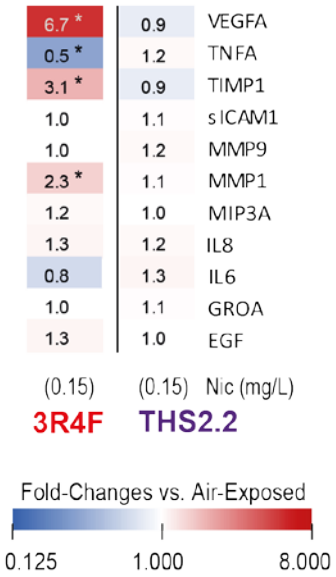


Ciliary Beating Frequency



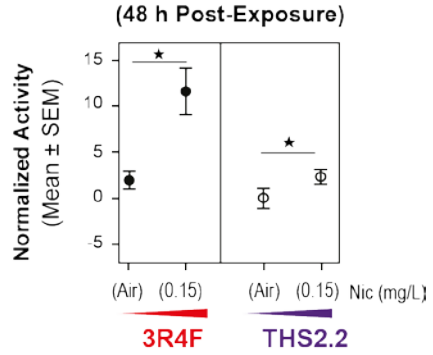
Alteration of Pro-Inflammatory Mediators Secreted into the Basolateral Media

(48 h Post-Exposure)



CYP1A1/1B1 Activity

(48 h Post-Exposure)



Alterations in FoxJ1-proportions, ciliary frequency, secreted pro-inflammatory mediator level, and activity of CYP1A1/1B1 were markedly lower following THS2.2 aerosol than 3R4F smoke at comparable nicotine concentration (0.15 mg/L)

PMI RESEARCH & DEVELOPMENT

A Series of Studies:
Cigarette Smoke (CS) Exposure Assessment using Organotypic Three Dimensional (3D) Upper and Lower Airway Epithelial Tissue Cultures

Author/ Year	Mode of Exposure	Duration	Cytotoxicity	Gene Expression	Cilia Frequency/ Function	Inflammatory Mediators	Epithelial Barrier Integrity	Additional endpoints
Mathis 2013	Single exposure to whole CS	7-28 min		✓		✓		miRNA profile
Iskandar 2013	Repeated exposure of whole CS	4 cigs		✓		✓		CYP activity
Talikka 2014	Repeated exposure of whole CS	4 cigs		✓		✓		Histology
Zhang 2014	Single exposure of CSE	1-4 h					✓	Glycosylation
Aufderheide 2015	Repeated exposure of whole CS	4 cig/d (8 d)	✓		✓			
Astrand 2015	Single exposure of whole CS	1 cig			✓			Epithelial sodium channel activity
Azzopardi 2015	Single exposure of whole CS	30 min	✓			✓		
Kuehn 2015	Repeated exposure of whole CS	4 cigs		✓	✓			CYP activity
Mathis 2015	Single exposure of whole CS	7-28 min		✓				miRNA profile
Iskandar 2015	Single exposure of whole CS	28 min	✓	✓		✓		Histology, CYP activity
Schamber 2015	Single exposure of CSE	7-28 d		✓	✓	✓		Protein expression
Zanetti 2016	Single exposure of whole CS and RRP	28 min	✓	✓		✓	✓	Histology, CYP activity, miRNA profile
Iskandar 2016	Single exposure of whole CS and RRP	28 min	✓	✓	✓	✓		Histology, CYP activity, miRNA profile

CSE: cigarette smoke extract. Studies conducted at PMI.

PMI RESEARCH & DEVELOPMENT

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1. Introduction
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5. Sbv IMPROVER

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Case study 3: Summary of ambulatory exposure clinical ZRHM-REXA-07-JP Study Results

This research was funded by Philip Morris International

Study Title

- A randomized, controlled, open-label, 3-arm parallel group, multi center study to demonstrate reductions in exposure to selected smoke constituents in healthy smokers switching to the Tobacco Heating System 2.2 Menthol (THS2.2 Menthol) or observing smoking abstinence, compared to continuing to use menthol conventional cigarettes, for 5 days in confinement and prolonged by 85 days in an ambulatory setting

Study Products and Interventions

= THSm2.2

(Tobacco Heating System 2.2 menthol)

= SA

(Smoking abstinence)

= mCC

(Menthol conventional cigarettes)

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Primary Objective and Endpoints

- To demonstrate the reduction of biomarkers of exposure (BoExp) to harmful and potentially harmful constituents (HPHCs) in smokers switching from menthol conventional cigarette (mCC) to THS2.2 Menthol (THSm2.2) compared to smokers continuing to smoke mCC
- MHBMA, 3-HPMA, S-PMA, COHb after 5 days (confinement)
- Total NNAL after 90 days (ambulatory)

Additional Objectives and Endpoints

- To determine the reduction of a list of BoExp and to determine the levels of nicotine over the entire exposure period
- To evaluate self-reported nicotine/tobacco product use and human smoking topography
- To describe product evaluation and subjective effects of smoking
- To monitor selected risk markers and the safety profiles

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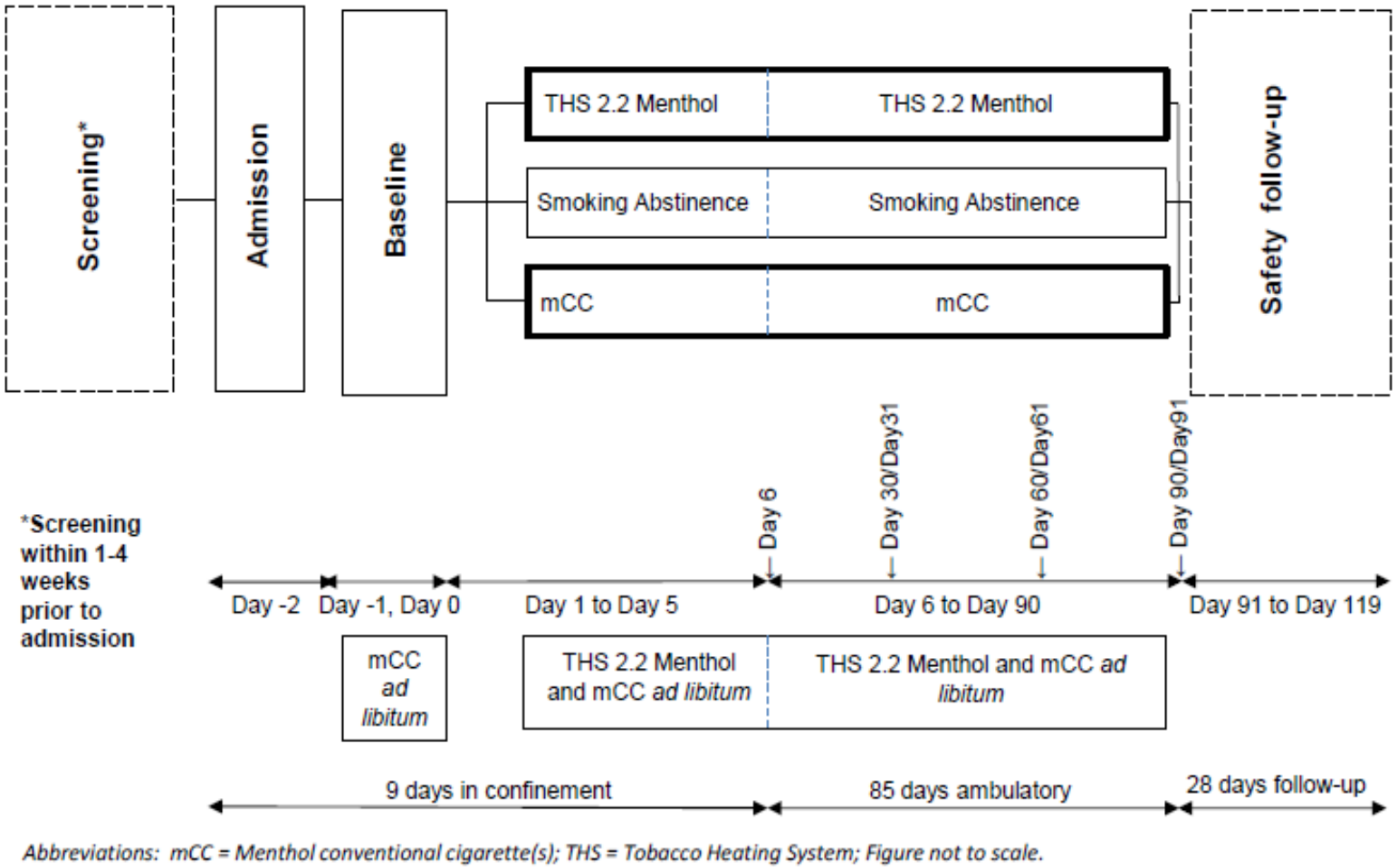
Study Design

Study Design



Abbreviations: mCC = Menthol conventional cigarette(s); THS = Tobacco Heating System; Figure not to scale.

Japanese Population Characteristics

Characteristics	THSm2.2 (N=78)	mCC (N=42)	SA (N=40)	Overall (N=160)
Females – n(%)	33 (42.3)	17 (40.5)	18 (45.0)	68 (42.5)
Age (years) - Mean±SD	37 ± 11	37 ± 11	37 ± 10	37 ± 11
BMI Normal Weight– n(%)	60 (76.9)	32 (76.2)	32 (80.0)	124 (77.5)
Daily mCC Consumption– n(%) 10-19 cig/day > 19 cig/day	 40 (51.3) 38 (48.7)	 23 (54.8) 19 (45.2)	 21 (52.5) 19 (47.5)	 84 (52.5) 76 (47.5)
ISO Tar yields – n(%) 1-5 mg 6-8 mg 9-10 mg > 10 mg	 46 (59.0) 21 (26.9) 7 (9.0) 4 (5.1)	 22 (52.4) 14 (33.3) 4 (9.5) 2 (4.8)	 23 (57.5) 12 (30.0) 2 (5.0) 3 (7.5)	 91 (56.9) 47 (29.4) 13 (8.1) 9 (5.6)
ISO Nicotine ≤ 0.6mg – n(%)	63 (80.8)	32 (76.2)	30 (75.0)	125 (78.1)

THSm2.2= THS 2.2 Menthol, mCC menthol Conventional Cigarettes, SA: smoking abstinence, SD: standard deviation

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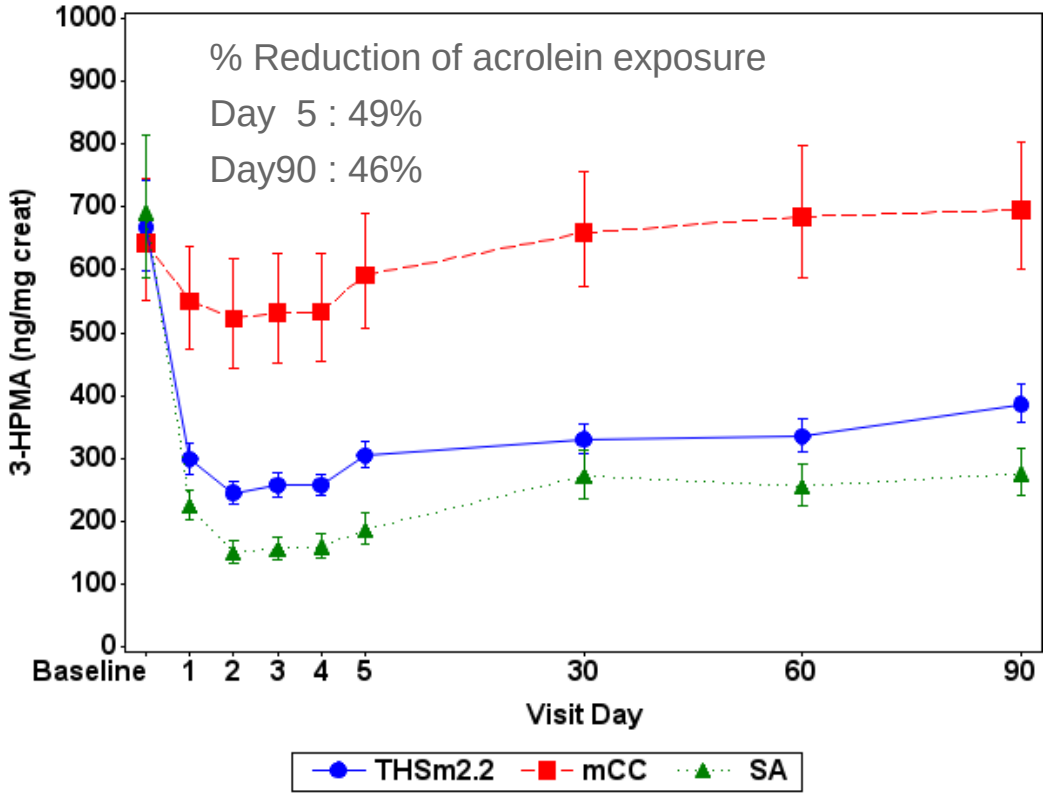
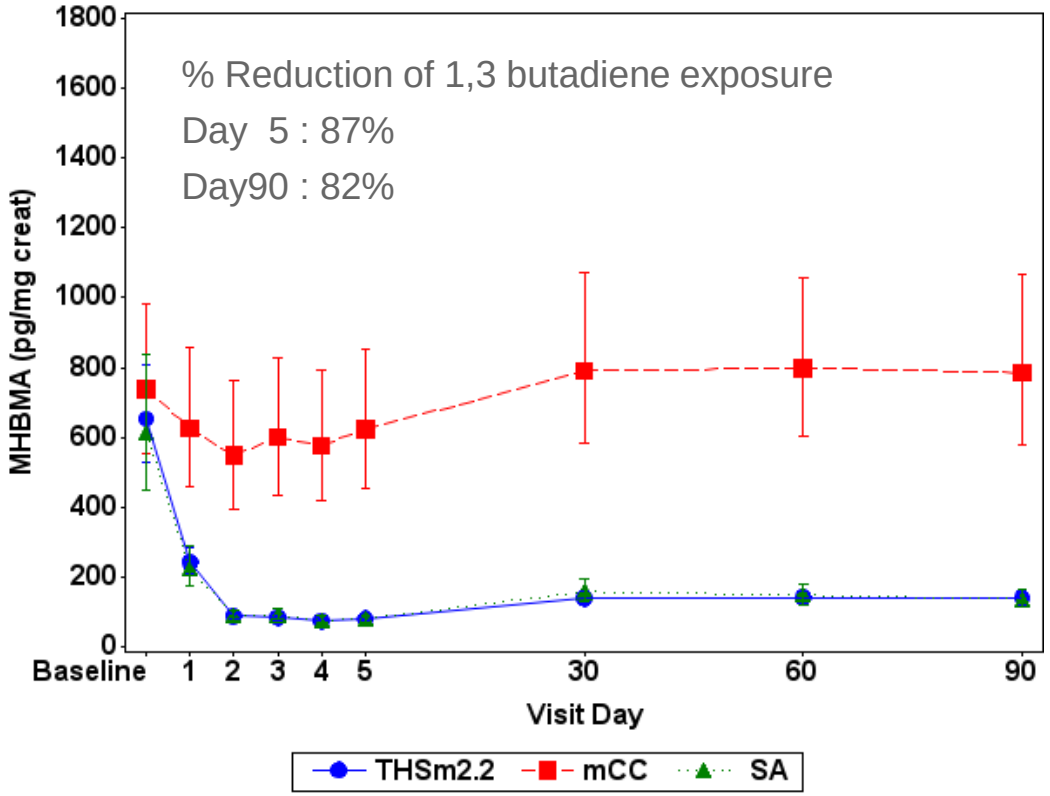
Exposure to Harmful and Potentially Harmful Compounds

Biomarkers following 90 days of exposure (1/3)





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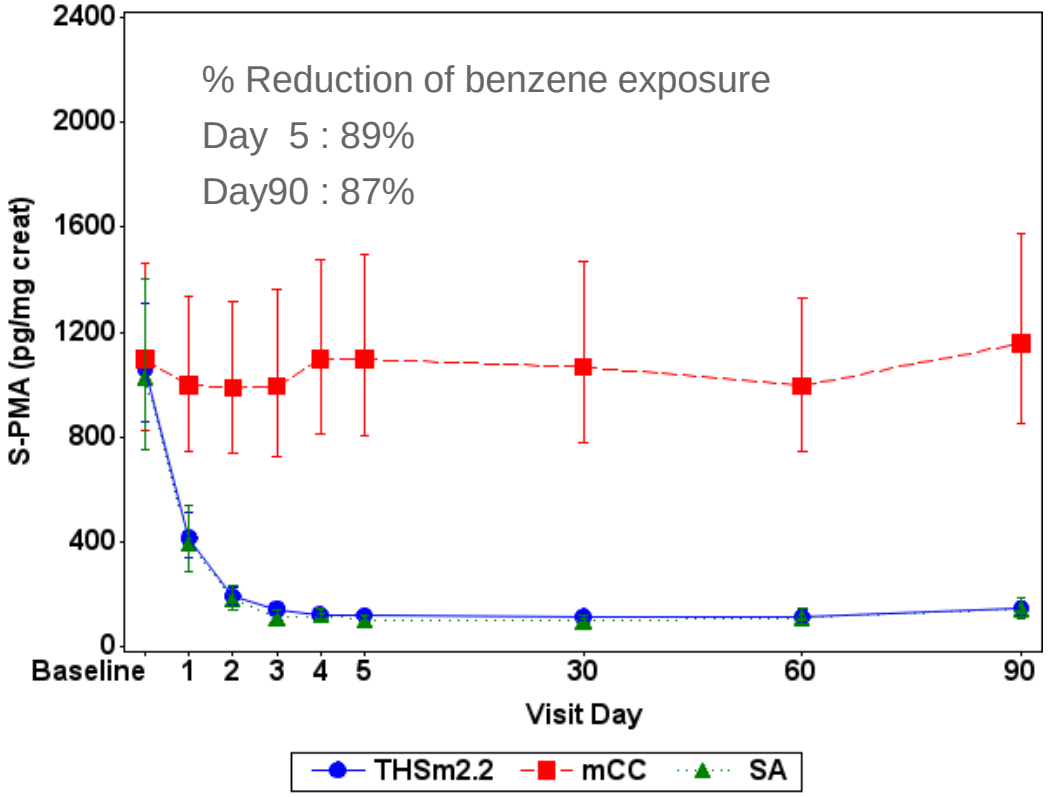
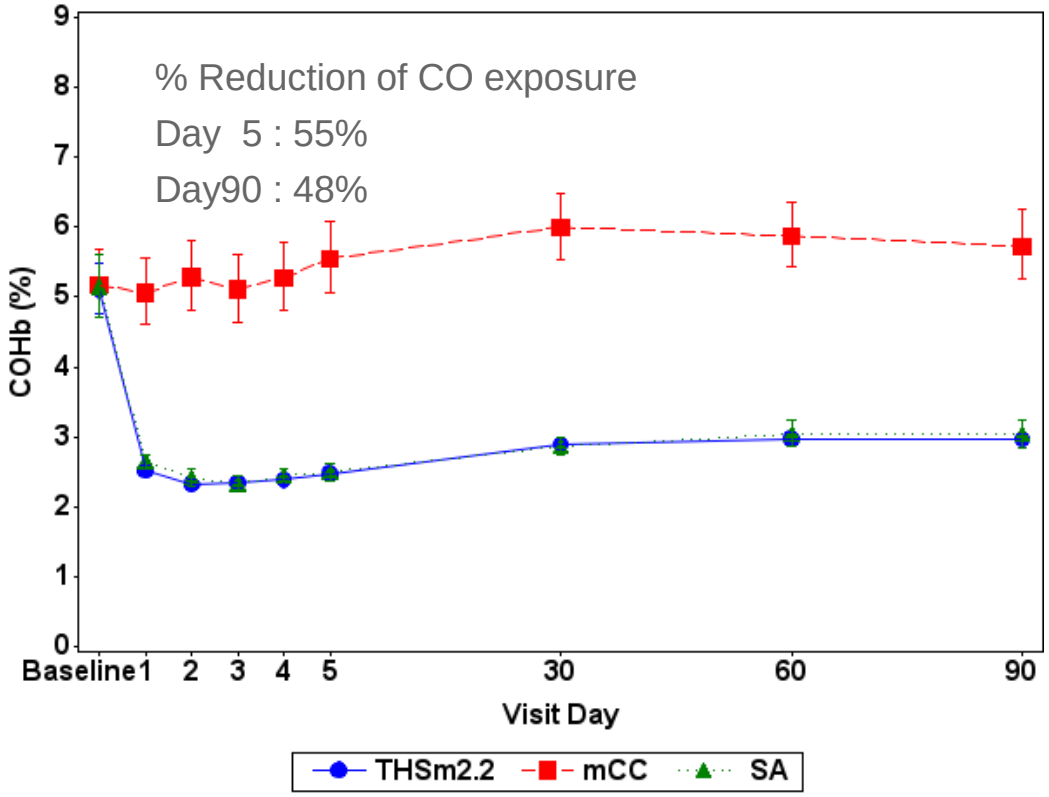
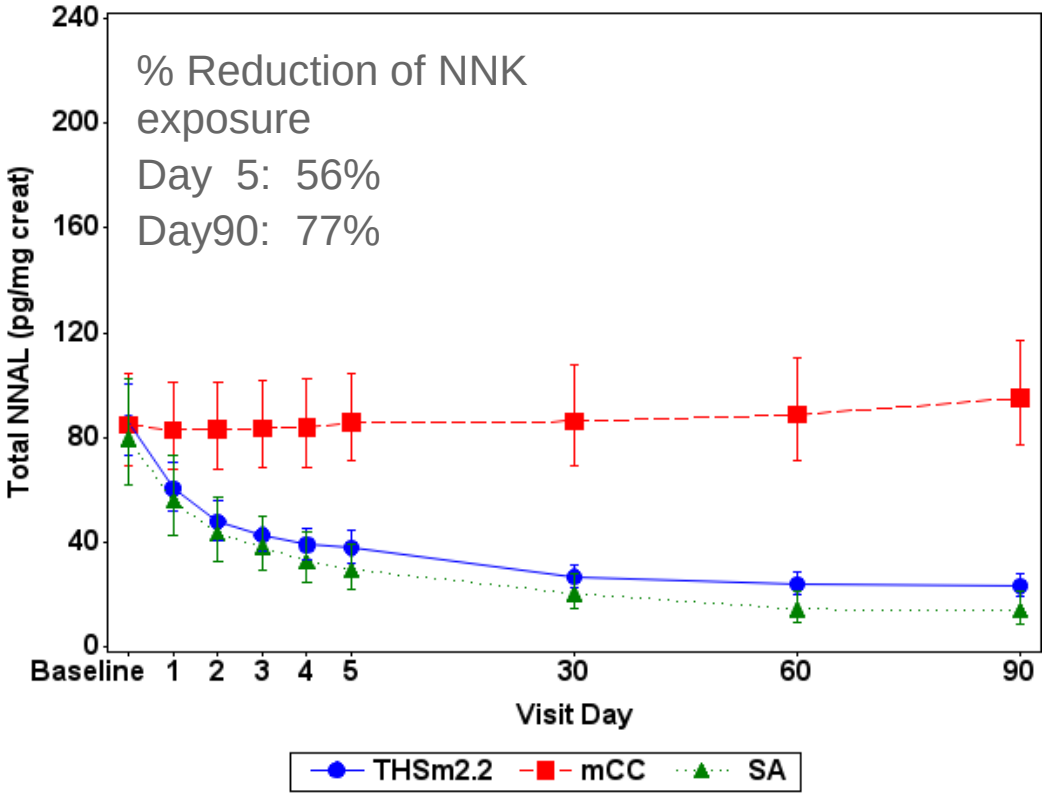
Biomarkers following 90 days of exposure (2/3)

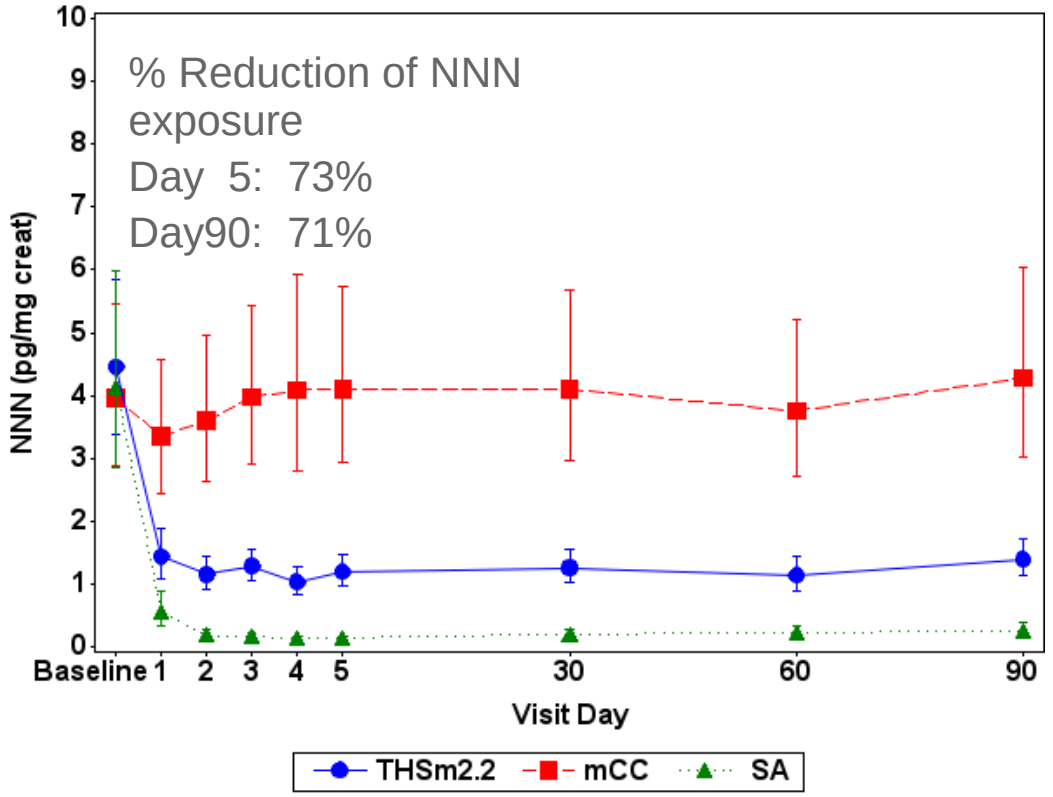




PMI RESEARCH & DEVELOPMENT

Biomarkers following 90 days of exposure (3/3)





PMI RESEARCH & DEVELOPMENT

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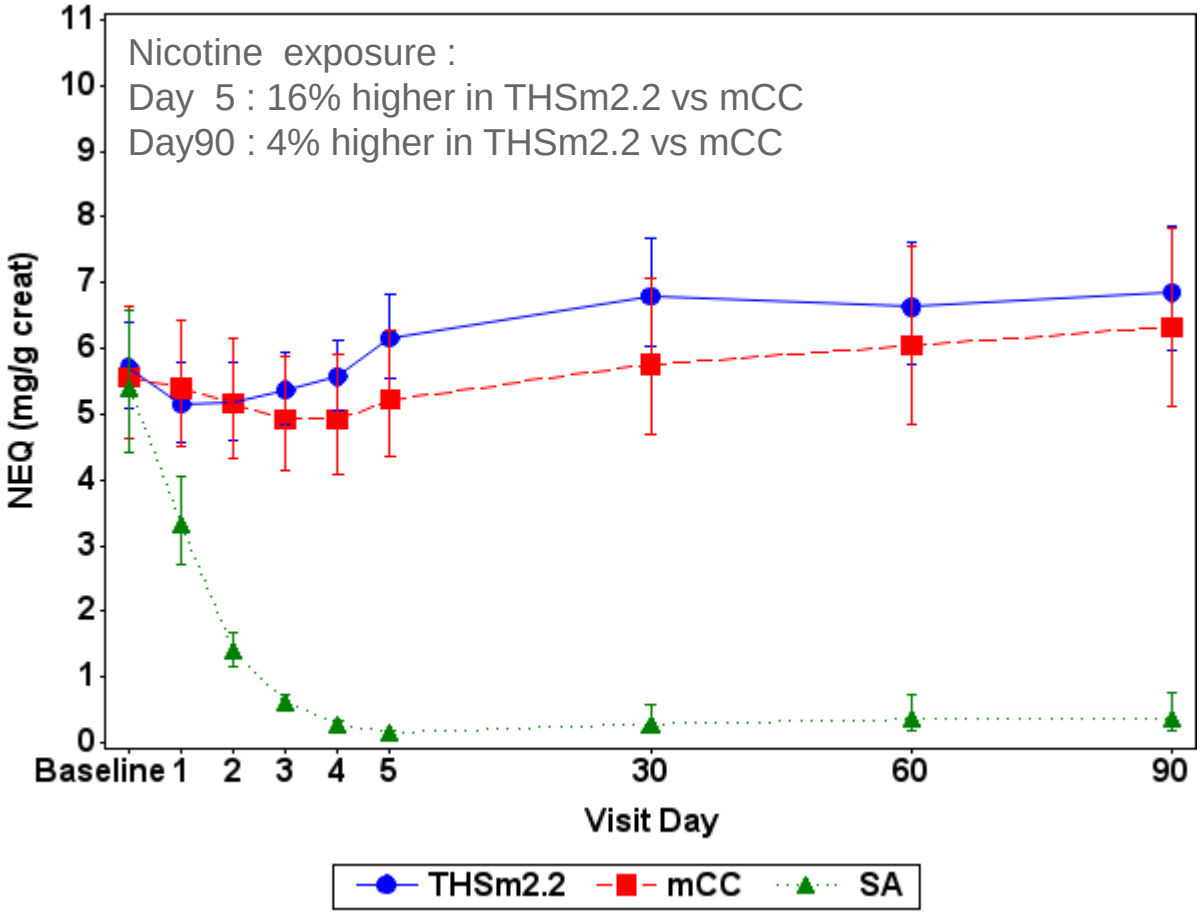
Product use

Product Use – Average Daily Product Use

Time point	THSm2.2 Mean±SD	mCC Mean±SD
Baseline mCC	13.1±3.8	12.5±3.9
Day 5	13.9±4.3	13.6±4.7
Day 5-30	11.7±5.9	13.8±4.2
Day 30-60	12.7±6.2	14.9±5.7
Day 60-90	12.7±6.5	15.2±5.0

THSm2.2= THS 2.2 Menthol, mCC menthol Conventional Cigarette, SD: standard deviation; n: subjects in the main analysis population (Per Protocol Set)

Nicotine Uptake



Exposure THSm2.2 vs. mCC

- Nicotine uptake profile is similar, marginally higher for THSm2.2
  - 80% of subjects smoke low-nicotine containing mCC.
- A process of adaptation is observed throughout the 3-month period, resulting in a 4% difference on day 90.
  - Nicotine yield in mCC variable, and fixed in THSm2.2

Selected Clinical Risk Endpoints (indicative results, not statistically significant)

Disease Pathway	Marker	Expected SA Effect at 6m(*)	REXA-07 SA Effect at 3m	REXA-07 THS Effect at 3m
Lipid Metabolism	HDL-C	4.13 mg/dL	6.4 mg/dL	4.5 mg/dL
Inflammation	WBC	-0.81 10^9/L	-0.41 10^9/L	-0.57 10^9/L
Airway Impairment	FEV_1	2.18 %pred	1.93 %pred	1.91 %pred
Endothelial Dysfunction	sICAM-1	20.0 %reduction	10.9 %reduction	8.7 %reduction
Oxidative Stress	8-epi-$PGF_{2\alpha}$	32.0 %reduction	6.0 %reduction	12.7 %reduction
Clotting	11-DTX-B_2	22.0 %reduction	19.4 %reduction	9.0 %reduction

(*) Expected SA effect from literature data

Safety

- No serious or severe adverse events (AEs).
- During the run-in (product test), 22 AEs observed in 16 (9%) of 175 enrolled.
- Following randomization, 49 AEs in 32 (41%) subjects in THS, 22 AEs in 14 subjects for both mCC (33%) and SA (35%) arms. One mild not expected AE related to product in THS arm (Diarrhea). Most frequent AEs were decreased hemoglobin and decreased neutrophils.
- No clinically relevant abnormality in vital signs, ECGs, or physical examination.

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Conclusion

Common conclusion

- All studies demonstrated that switching from mCC smoking to THSm2.2 aerosol resulted in substantial reductions in exposure to selected HPHCs.
- Reduction following switching to THSm2.2 achieved HPHC levels close to those observed following smoking abstinence (SA).
- Initial exploratory clinical data on monitored risk markers indicate favorable shifts in the direction of SA. While in ApoE-/- mice, switching from cigarette smoke exposure to THS2.2 aerosol exposure reverses the onset of disease in a similar manner as cessation

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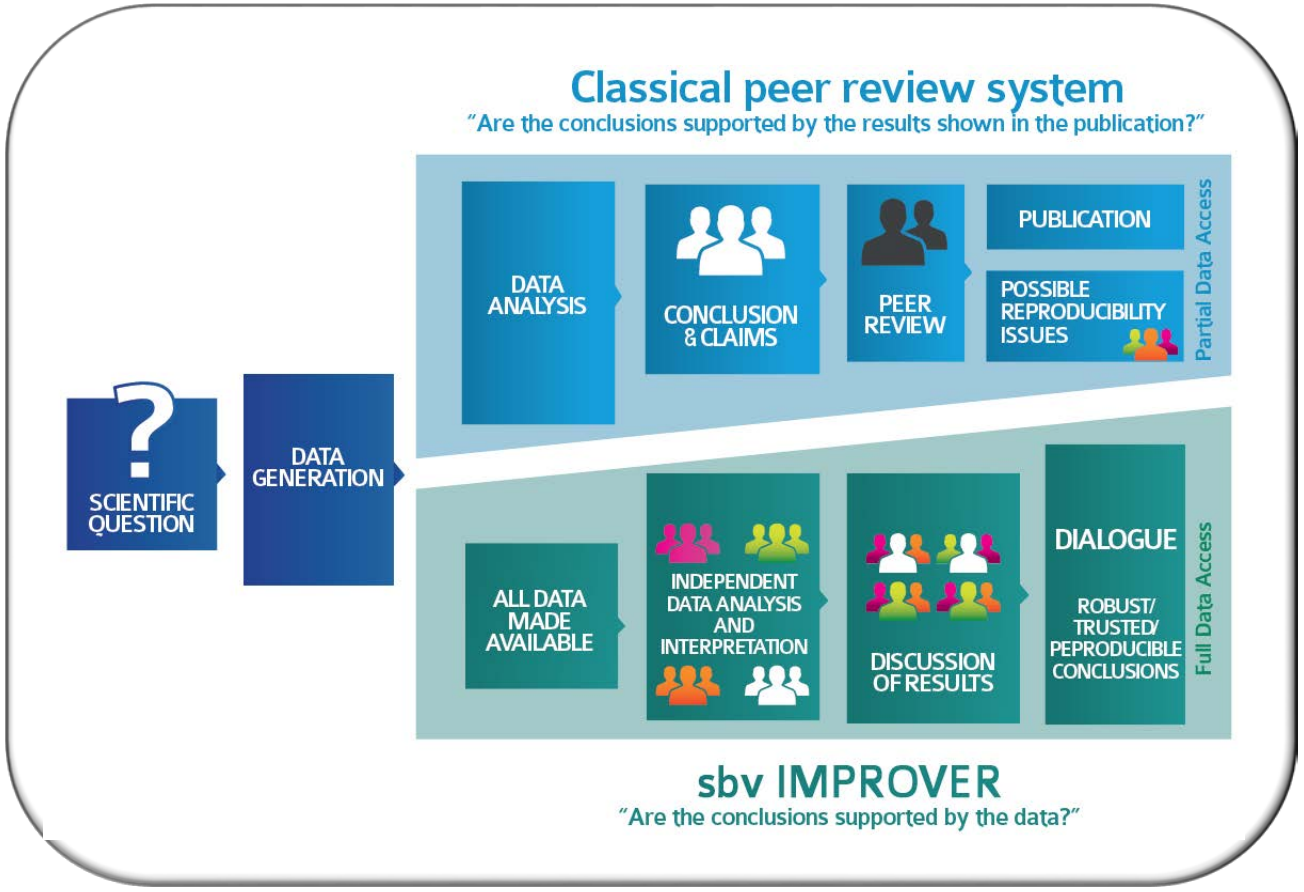
sbv IMPROVER Leverages the Crowd to Complement the Classical Peer Review System



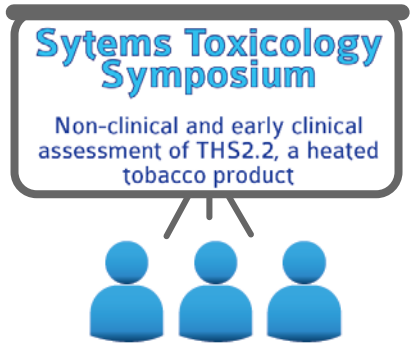
- **Crowd-sourcing**: A natural evolution of web technologies led to the development of distributed problem-solving. Challenges are broadcasted to potential interested stakeholders (solvers). The winning participants are rewarded either with monetary awards, prizes, certificates, or with recognition.
- **Collaboration by Competition**: The scientific community sought to understand the limitations and comparative advantages of their methods by challenging model developers to make blind predictions on previously unseen data in a competitive framework.
- The community appreciates the successful methods which grow in credibility. Therefore, consideration of the scientific community is one of the forces that shape what is **currently considered as the way to do the science right**

Meyer et al: Verification of systems biology research in the age of collaborative competition. Nature biotechnology 2011, 29 (9), 811.
Meyer et al: Industrial methodology for process verification in research (IMPROVER): toward systems biology verification. Bioinformatics 2012, 28 (9), 1193-1201.

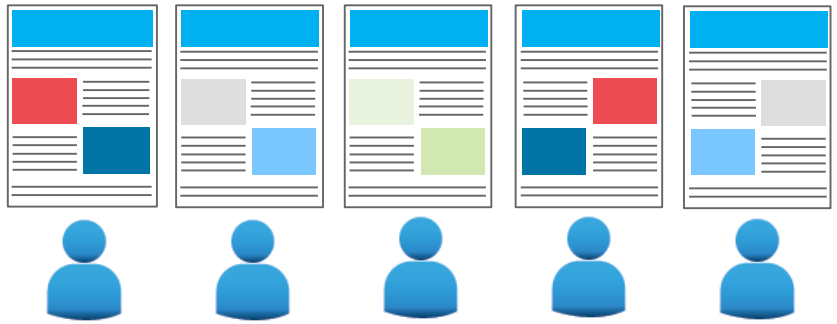
sbv IMPROVER Leverages the Crowd to Complement the Classical Peer Review System

Poster session™ - ACT 2016

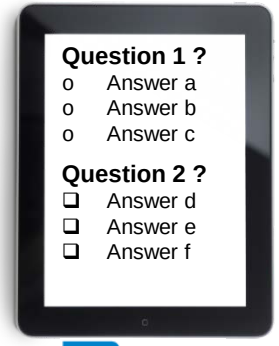
1. Intro Presentation 7:30



2. Poster Review 8:00



3. SciPi™ Survey 8:00



SciPinion

4. Results & Discussion 9:00

5. Networking 9:30

PMI RESEARCH & DEVELOPMENT

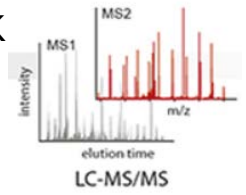
Thank You For Your Attention !

Peitsch, M; Hoeng J; Vanscheeuwijck, P; Luettich, K, Ee Tsin
(Study Design)

Oviedo, A (Animal treatment)
Krishnan, S (Aerosol)
Seow, E (Bioanalytics, dissection and histoprocessing)
Bundularatne,E & Ng,G (Veterinarian)
Ansari, S (Biobanking)

Ivanov N
Research Technologies

Transcriptomics
Guedj, E
Baumer, K
Dulize, R
Peric, D
Bonard, D

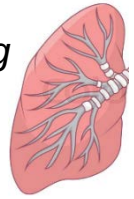


Proteomics
Elamin, A
Nury, C
Schneider, T
Dijon, S
Titz B

Genomics
Sierro, N
Ouadi, S
Thomas, J
Batty, J

sbv IMPROVER
SYSTEMS BIOLOGY VERIFICATION

Tissue Processing
Trivedi, K
Benyagoub, A



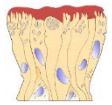
Computational Analysis
Martin, F
Xiang, Y
Titz, B
Talikka M

PMI high performance computing

PMI cellular labs
Frentzel, S, Iskandar A, Majeed S, Zanetti F et all.

Statistics
Leroy, P
Gregory, V

PMO team, Betch P, Lebrun S, Schilli L, Ertan A



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Thank you for your attention