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Six-month systems toxicology inhalation/cessation study in ApoE$^{-/-}$ mice to investigate cardiovascular and respiratory exposure effects of two reduced-risk products compared with cigarette smoke

1st Scientific Summit on Tobacco Harm Reduction: Novel Products, Research & Policy

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PMI R&D
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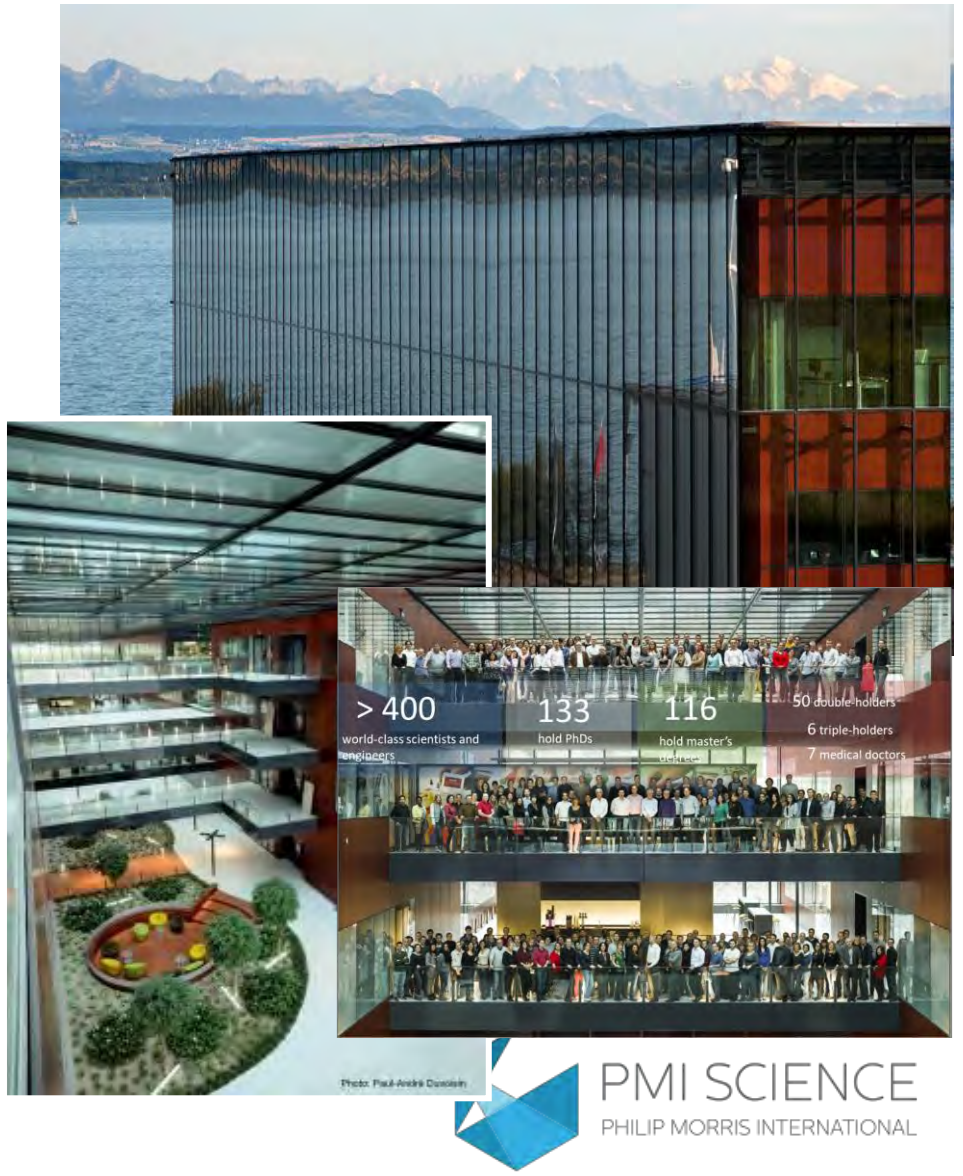
Background to PMI R&D

- Smoking causes serious diseases, such as cardiovascular disease, lung cancer, and chronic obstructive pulmonary disease.
- Philip Morris International is developing, assessing, and commercializing a number of Reduced-Risk Products* that have the potential to present less risk of harm compared with smoking cigarettes.
- Scientific determination of the reduced risk potential of these products includes comparison of the biological impact with that of a 3R4F reference cigarette on a mechanism-by-mechanism basis.



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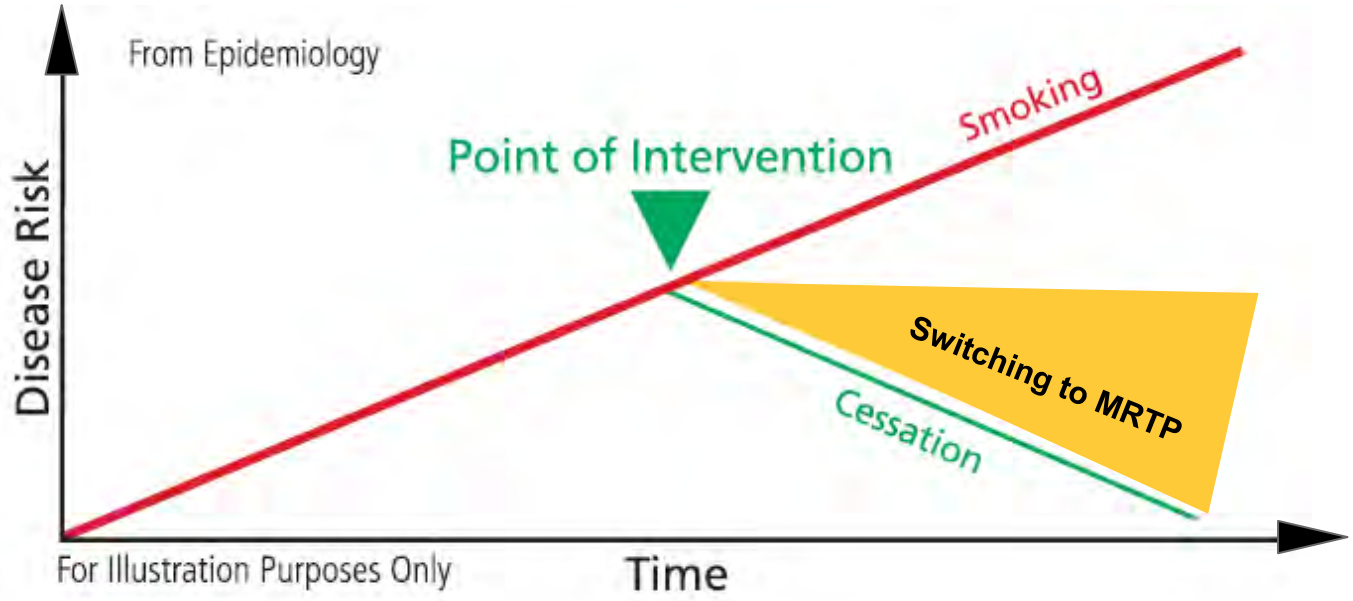
* Reduced-Risk Products ("RRPs") is the term we use to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to these products versus continued smoking. We have a range of RRPs in various stages of development, scientific assessment, and commercialization. Because our products do not burn tobacco, they produce far lower quantities of harmful and potentially harmful compounds than found in cigarette smoke

- Smoking is addictive and causes a number of serious diseases
- Worldwide, it is estimated that more than one billion people will continue to smoke in the foreseeable future*

- Successful harm reduction requires that current adult smokers be offered a range of Reduced-Risk Products so that consumer acceptance can be best fulfilled
- Our ambition is to lead a full-scale effort to ensure that noncombustible products ultimately replace cigarettes to the benefit of adult smokers, society, our company, and our shareholders

Risk Framework for MRTP Assessment



MRTP: Modified-Risk Tobacco Product

- Compare switching to a candidate MRTP with continued smoking and benchmark against smoking cessation (= "gold standard" as defined by U.S. Institute of Medicine)
- Assess how close switching to candidate MRTP is to smoking cessation

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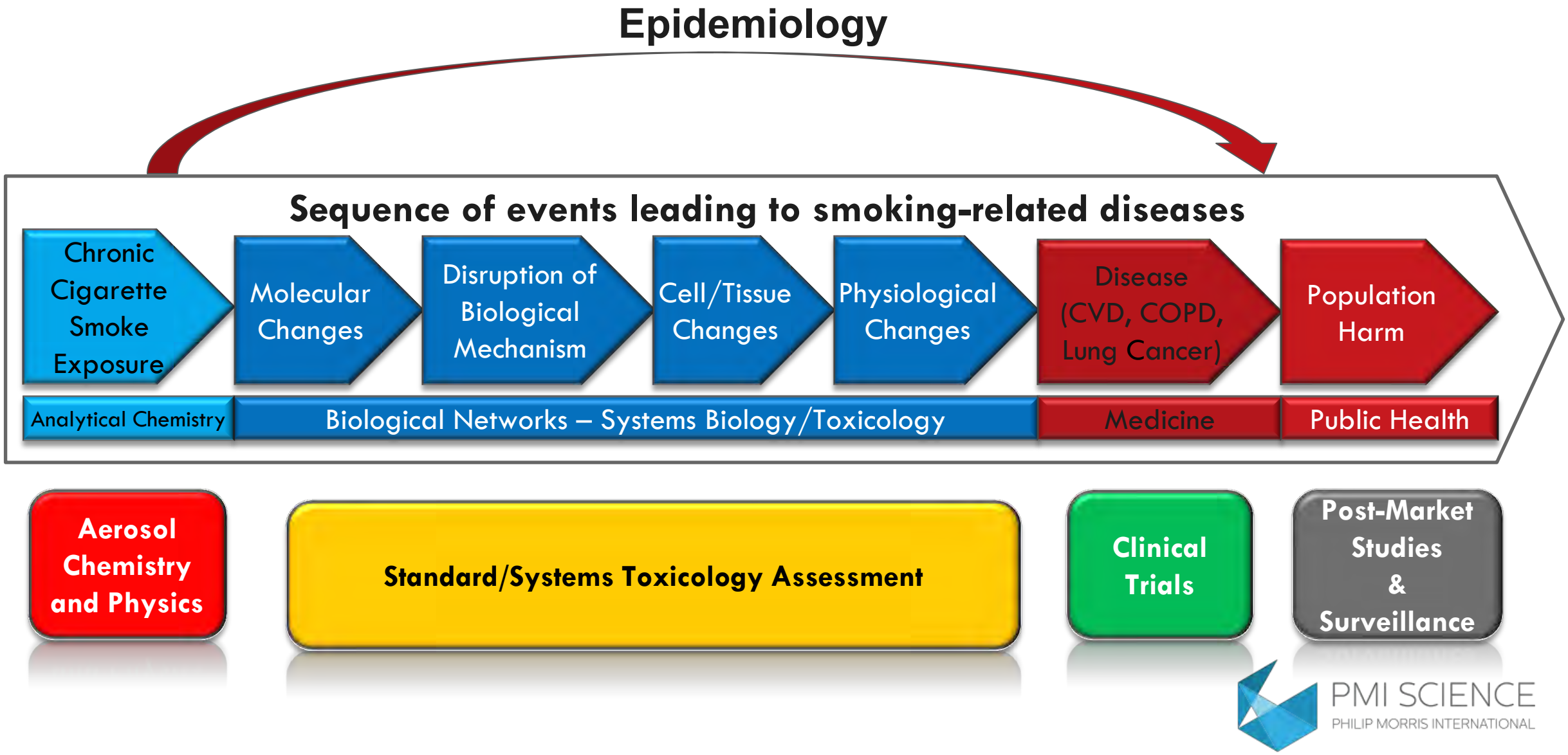
Source: IOM (Institute of Medicine), 2012, Scientific Standards for Studies on Modified Risk Tobacco Products. Washington, DC: The National Academies Press

From Chronic Exposure to Population Harm: A Causal Chain of Events



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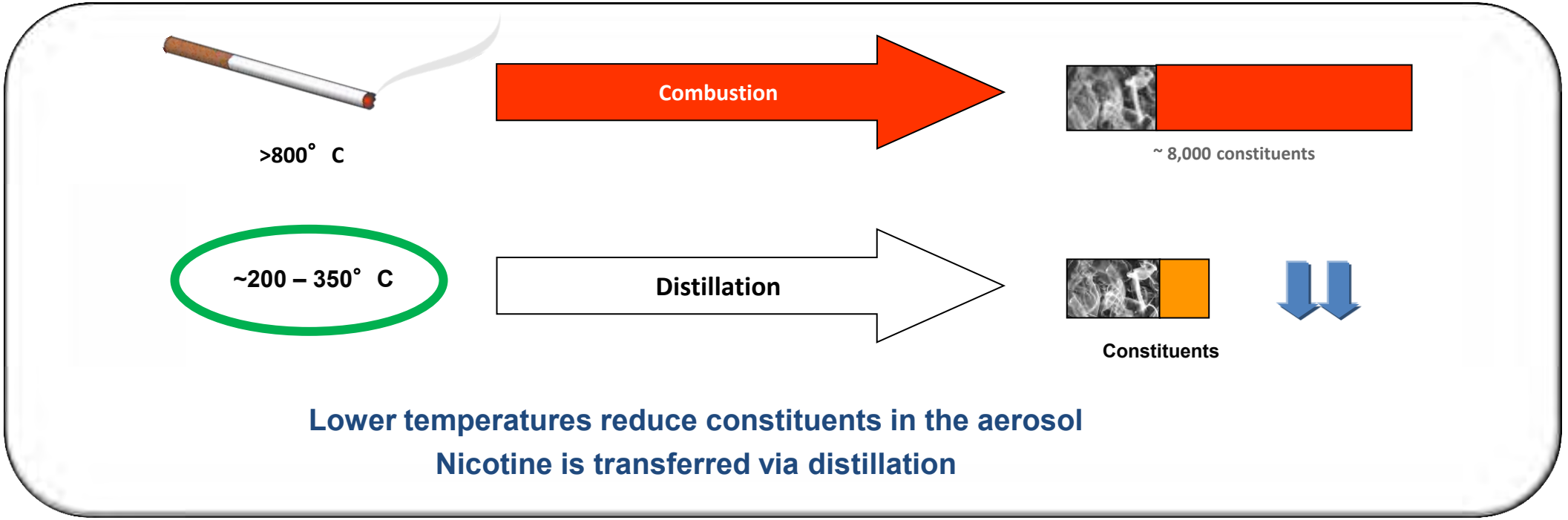
Smith, M. R., et al. (2016). "Evaluation of the Tobacco Heating System 2.2. Part 1: description of the system and the scientific assessment program." Regulatory Toxicology and Pharmacology 81: S17-S26.

Underlying Principles

- Approximately 8,000 constituents identified in cigarette smoke
- Some of these constituents are categorized as harmful and potentially harmful (HPHC)
- 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 is not an effective approach



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THS 2.2 – Operating Principles (IQOS Commercial Product)

Key Principles:

- Electrically heated tobacco system version 2.2 (THS 2.2)
  - Tobacco plug
  - Tobacco blends and flavor systems developed to suit lower operating temperature (< 350° C)

- Heating engine controlled precisely using built-in software
  - Tobacco is heated in a controlled fashion rather than burned, which is intended to prevent generation of HPHCs through pyrogenesis and pyrosynthesis
  - Heater also acts as a temperature sensor

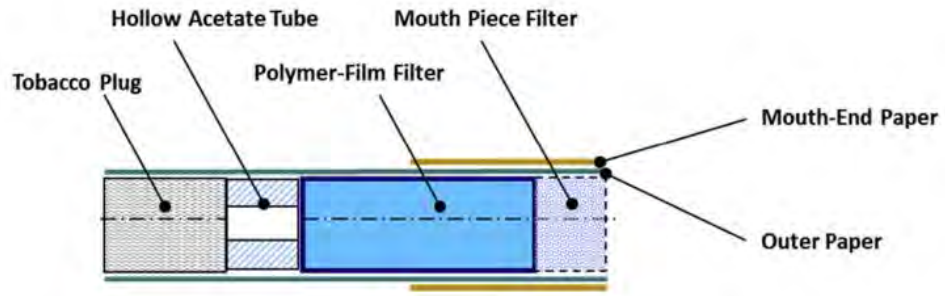

Fig. 3. Cross-sectional view of the *tobacco stick*.

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

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

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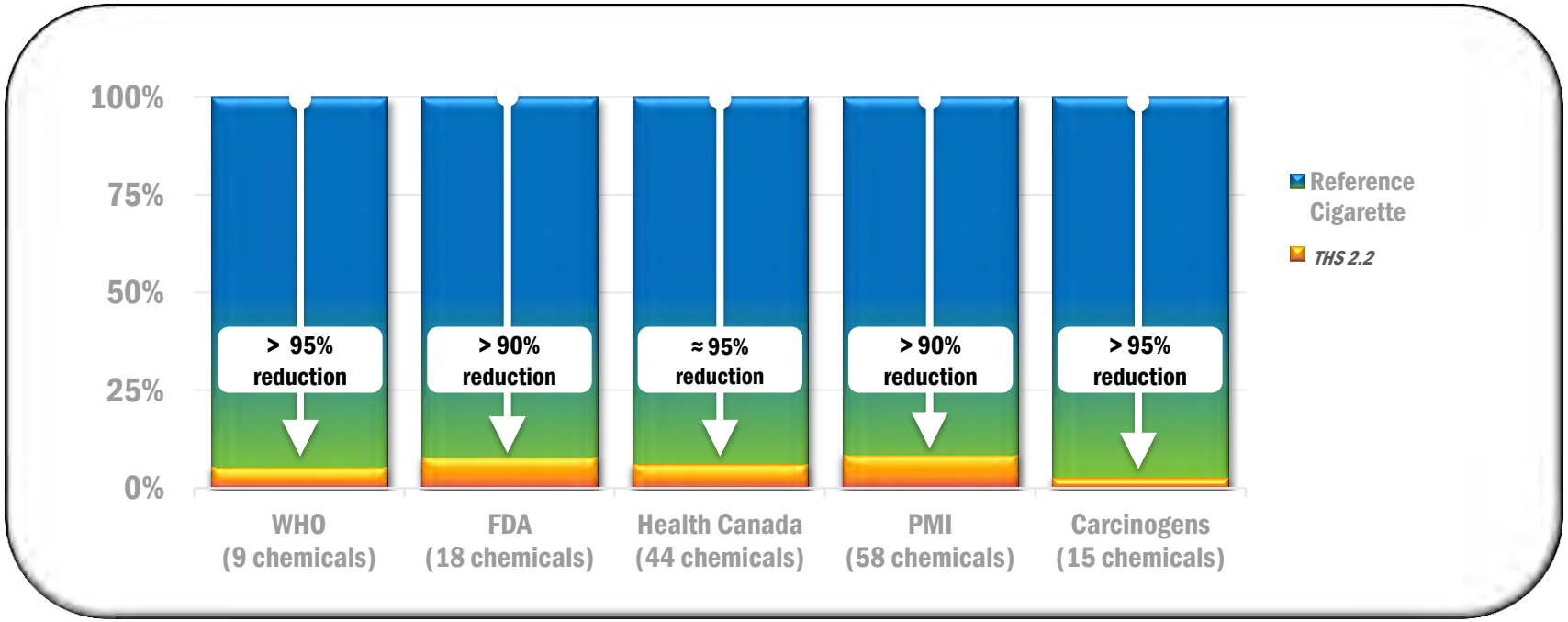
Average reductions in formation of HPHCs for THS 2.2 compared with levels measured in smoke from the 3R4F reference cigarette*



*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.

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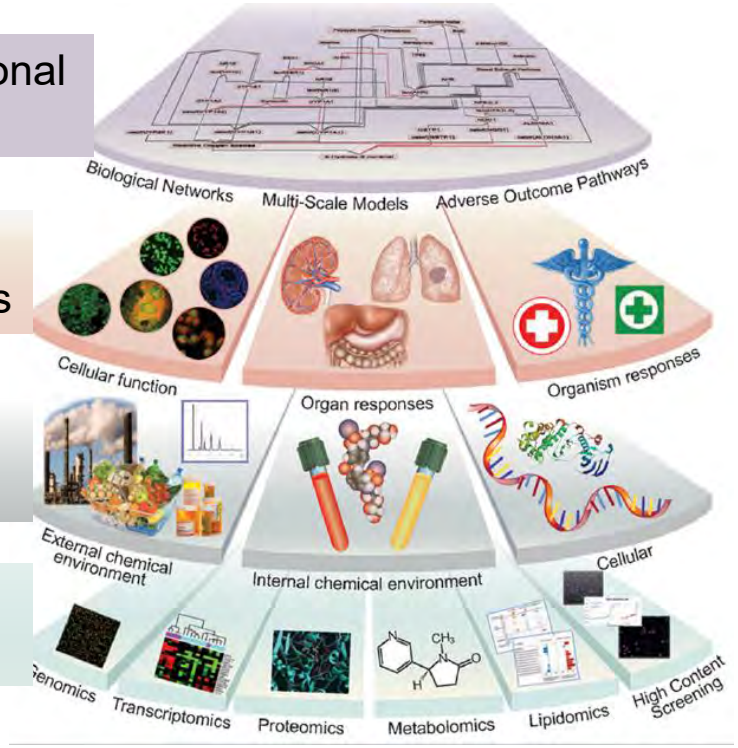
- Decoding the toxicological blueprint of active substances that interact with living systems
- Integrates classic toxicology approaches with network models and quantitative measurements of molecular and functional changes occurring across multiple levels of biological organization

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

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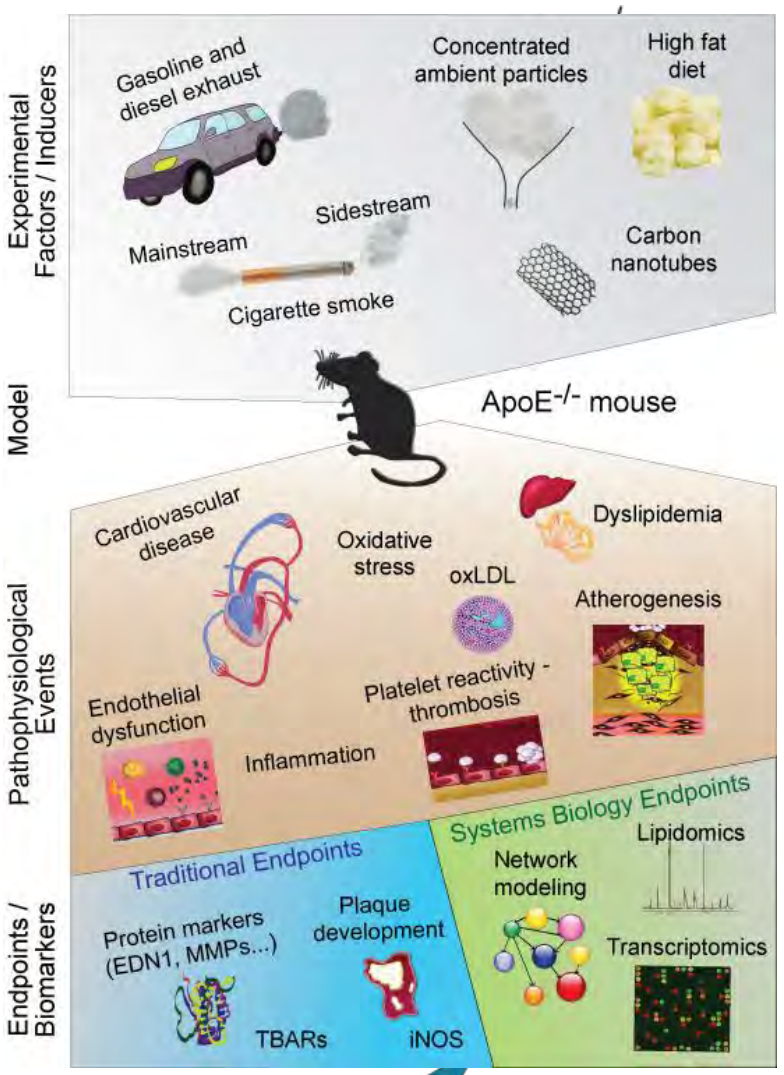
Sturla SJ, Boobis AR, Fitzgerald RE et al. (2014) Systems Toxicology: from basic research to risk assessment. Chemical research in toxicology 27:314-329

Smoking and CHD & COPD

- Atherosclerosis is an inflammatory disease characterized by the accumulation of lipoproteins and leukocytes as plaques in the arterial intima. Uncontrolled, it can lead to coronary heart disease (CHD) and underlying clinical events such as heart attack or angina.
- Development of CHD is accelerated by a variety of risk factors, including male gender, smoking, dyslipidemia, elevated blood pressure, physical inactivity, obesity, and diabetes.
- Patients with chronic obstructive pulmonary disease (COPD) have increased cardiovascular morbidity and mortality.
- ApoE$^{-/-}$ mice are the most widely used pre-clinical model of atherosclerosis.
- ApoE$^{-/-}$ mice show delayed lipoprotein clearance and consequently develop hyper- and dyslipoproteinemia, severe hypercholesterolemia, and atherosclerotic lesions, even when on a normal diet



Sasso, G. L., et al. (2016). "The Apoe−/− mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction." Journal of translational medicine 14(1): 146.

Herrington, W., et al. (2016). "Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease." Circulation research 118(4): 535-546.

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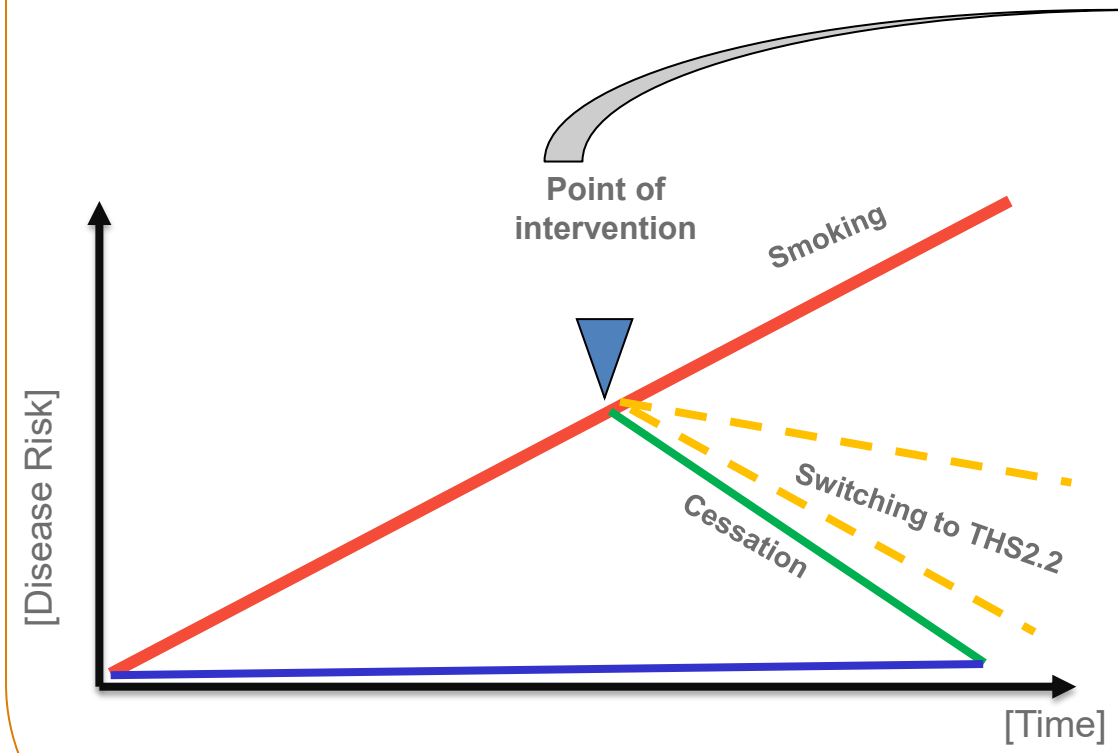
Switching Study in an Animal Model of Disease

Does switching from 3R4F cigarettes to THS 2.2 halt or delay the progression of vascular and respiratory pathologies?



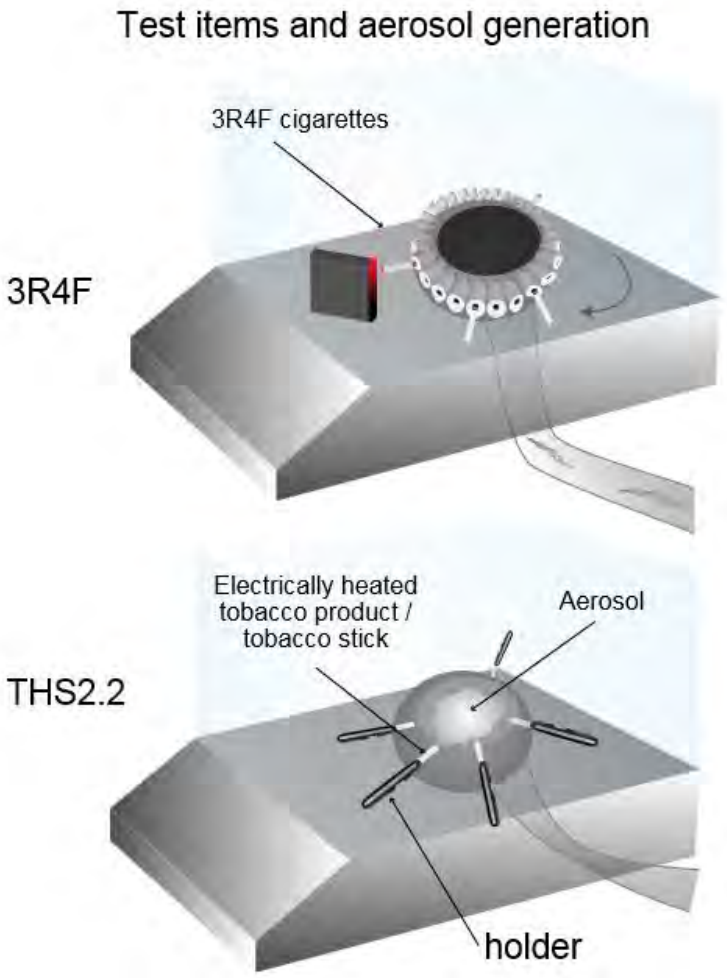
Mouse models of disease

Cardiovascular disease	COPD
Apoe$^{-/-}$ mice	Apoe$^{-/-}$ & C57BL/6 mice

Compare switching to THS 2.2 with ongoing smoking and benchmarking against cessation

Month	1	2	3	6	8	# animals BALF	Histo-pathology	Lung Function	Omics	Micro-CT	TOTAL 828
Sham						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8	6	44
3R4F						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8	6	44
THS2.2						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8	6	44
Cessation						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8	6	44
Switch						10	10	10	8		38
						10	10	10	8		38
						10	10	10	8	6	44

Aerosol Generation & Test Atmosphere



- Mice were exposed to 3R4F aerosol at a concentration of 600 mg TPM/m^3, equivalent to 29.9 µg nicotine/l, for 3 hours/day, 5 days/week. The dose of THS 2.2 was matched to the same nicotine level.
- Based on the most conservative approach, based on body surface extrapolation provided by the FDA this is equivalent to **~30 cigarettes (with a 1 mg nicotine per cigarette) per day for a 60 kg adult smoker.**

Test atmosphere	Sham	3R4F	THS2.2
TPM (mg/m^3)	0 (250)	598.5 ± 27.1 (256)	368.9 ± 54.4 (250)
Nicotine (mg/m^3)	0 (63)	29.4 ± 2.2 (256)	28.6 ± 3.2 (250)
Carbon monoxide (ppm)	0.1 ± 0.1 (250)	650.9 ± 34.2 (256)	14.5 ± 2.2 (250)
Acetaldehyde (mg/m^3)	n.d.	32.9 ± 2.3 (50)	7.5 ± 0.7 (49)
Acrolein (mg/m^3)	n.d.	3.1 ± 0.2 (50)	0.3 ± 0.0 (49)
Formaldehyde (mg/m^3/l)	n.d.	0.6 ± 0.1 (50)	0.1 ± 0.0 (49)

Data are shown as mean ± SD, number in brackets represents sample number (n).

Values exclude the first 7 days (dose adaptation). n.d., not done.

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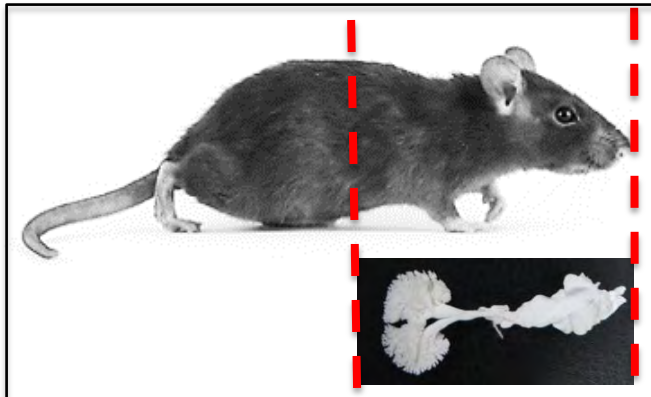
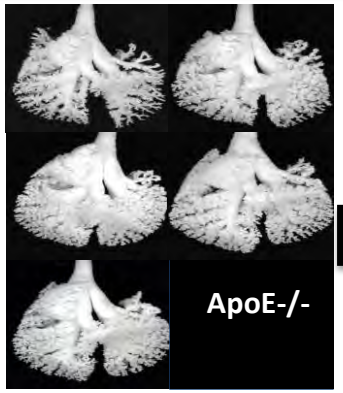
Characterization of 3R4F, THS 2.2, and Carbon Heated Tobacco Product (CHTP) 1.2 Aerosol Particles

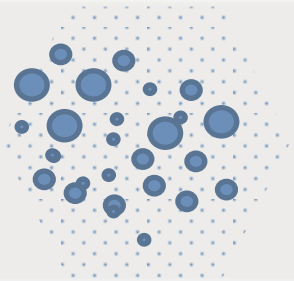


ApoE$^{-/-}$ mice respiratory system

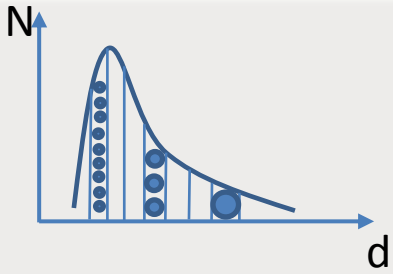
~ 1 cm



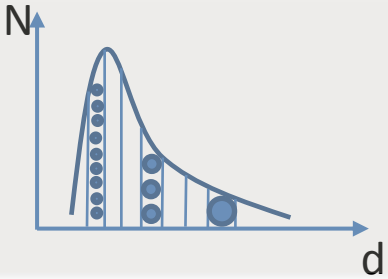
Lung cast



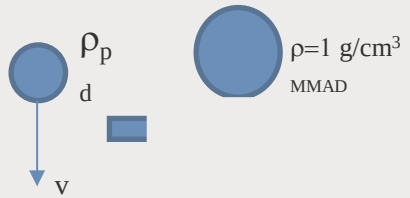
Aerosol: solid/liquid particles suspended in a gas atmosphere



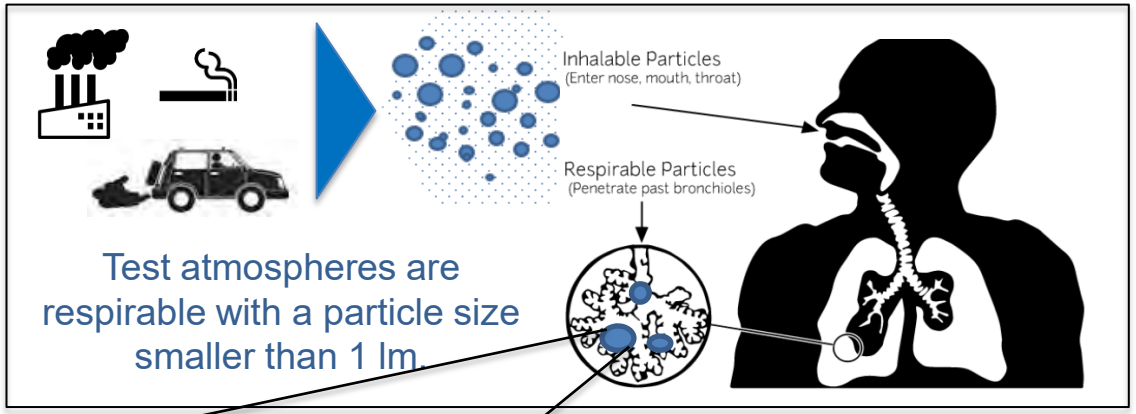
Discrete aerosol characterization:
- Particle number density (N): how many particles in a volume?
- Diameter (d): their size?



Assuming log-normal distribution parametrized via:
- **Geometric size distribution (GSD): width**
- **Mass median aerodynamic diameter (MMAD):** representative size

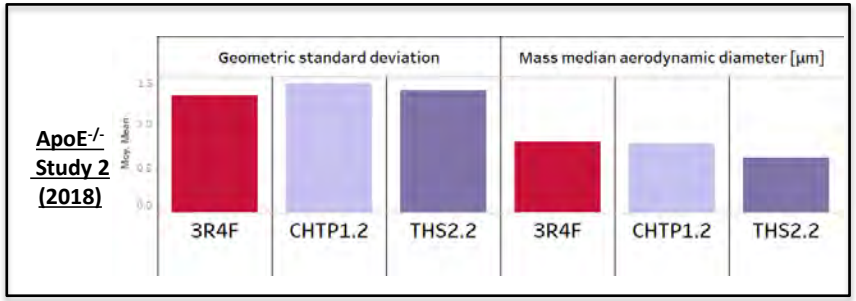


Aerodynamic diameter: Equivalence to spherical water droplet having the same settling velocity (v)

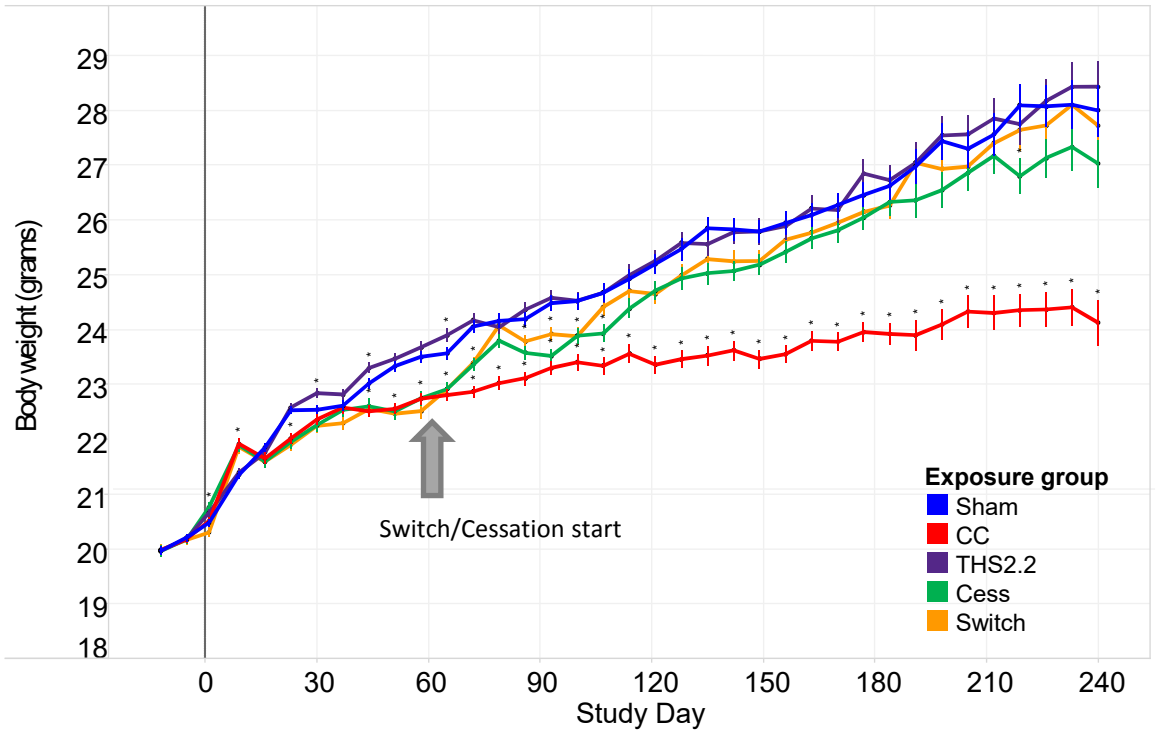


Test atmospheres are respirable with a particle size smaller than 1 lm.

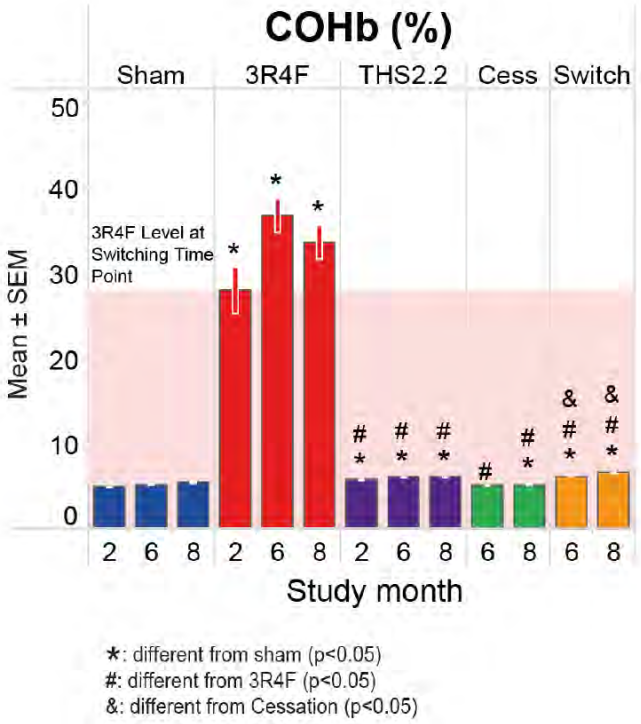
Measure aerosol size parameters in response to 3R4F CS or THS 2.2, CHTP 1.2

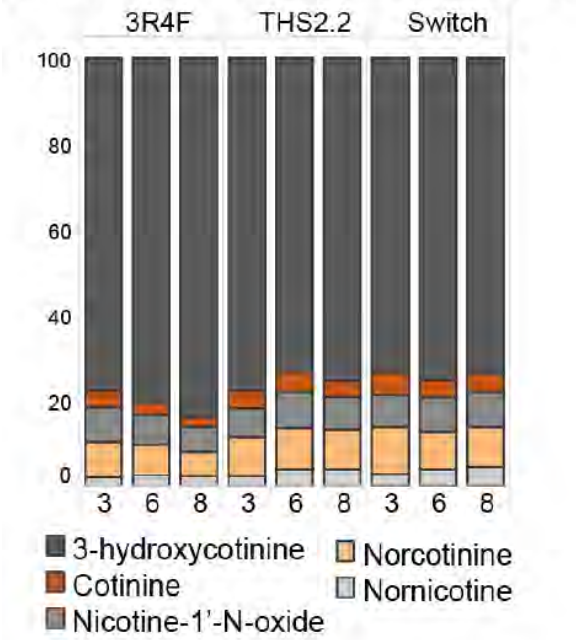


Body weight progression



Biomarkers of exposure





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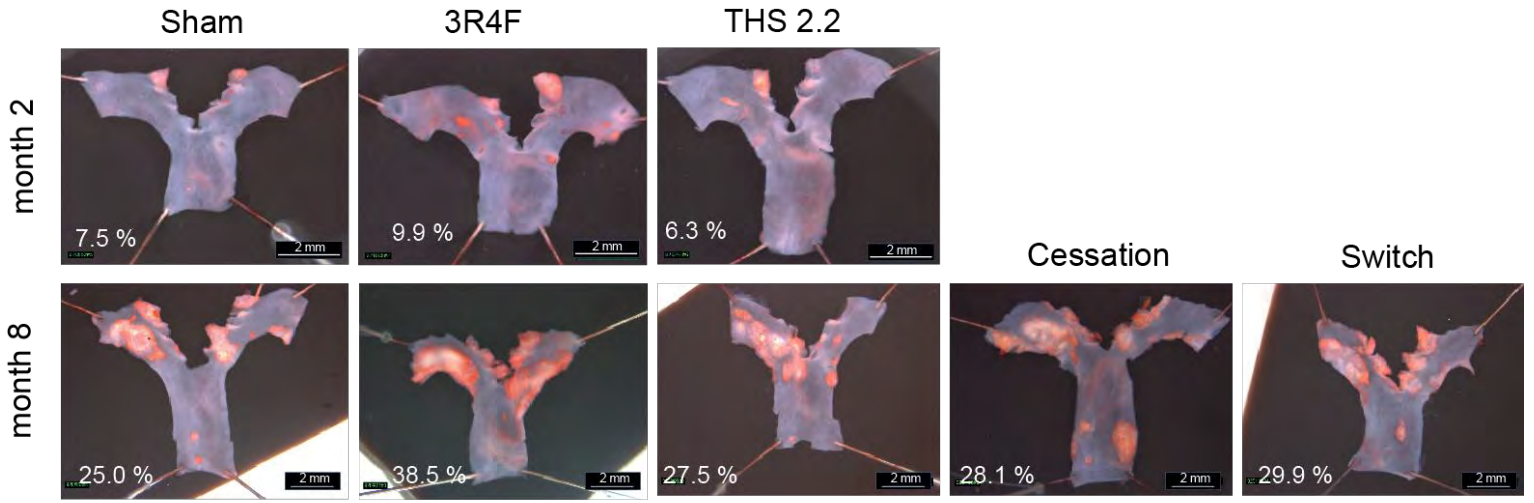
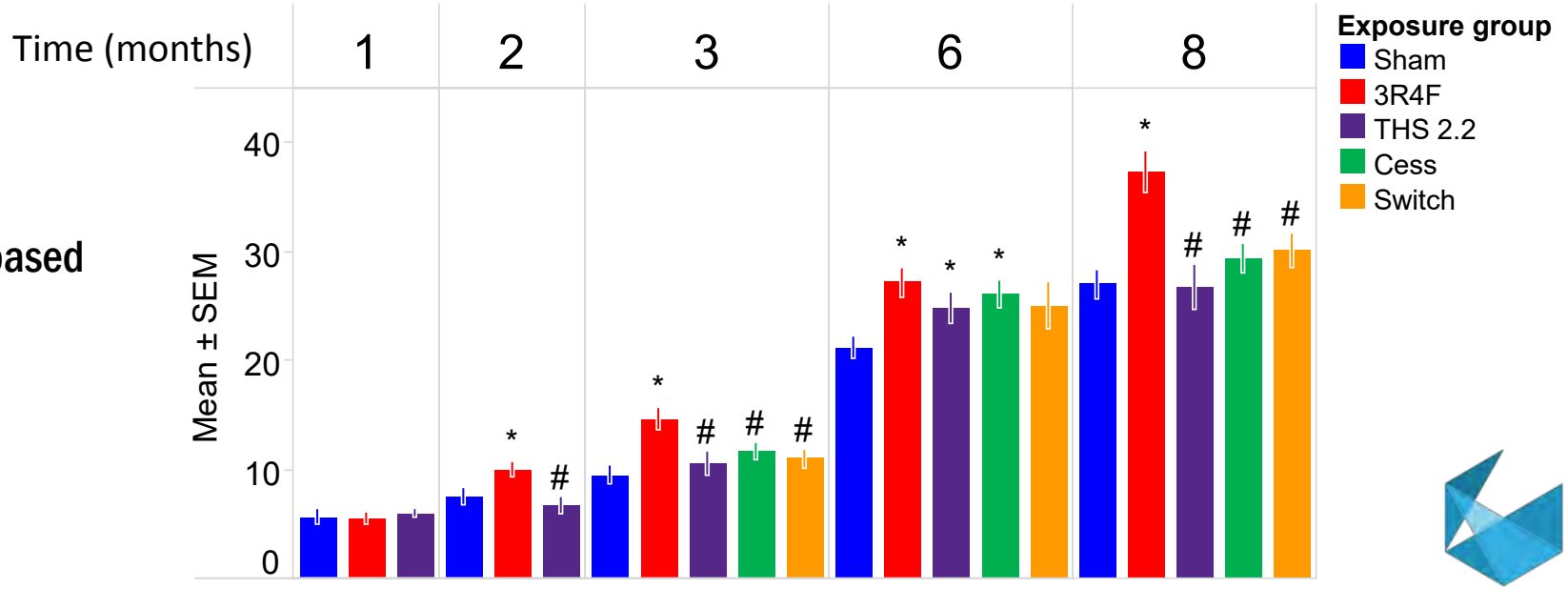
Phillips, B., et al. (2015). "An 8-month systems toxicology inhalation/cessation study in Apoe−/− mice to investigate cardiovascular and respiratory exposure effects of a candidate modified risk tobacco product, THS 2.2, compared with conventional cigarettes." Toxicological Sciences 149(2): 411-432.

Disease Endpoint - Aortic Plaque Growth

Example images of stained plaque in aorta



Percentage of plaque based on mm2



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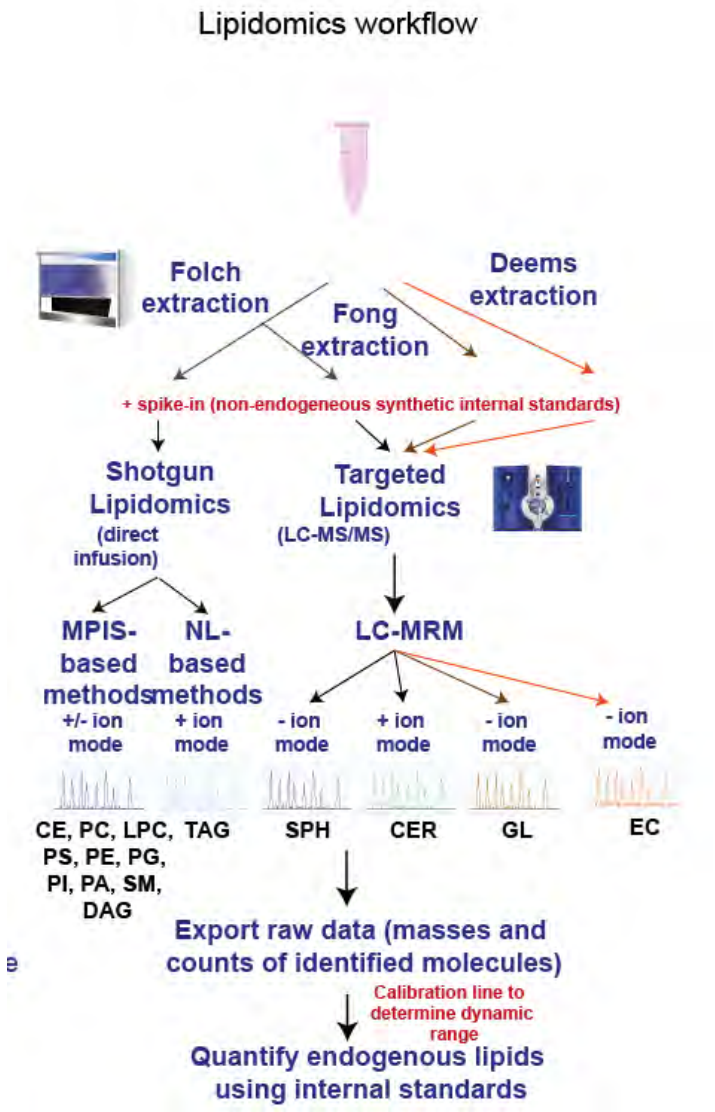
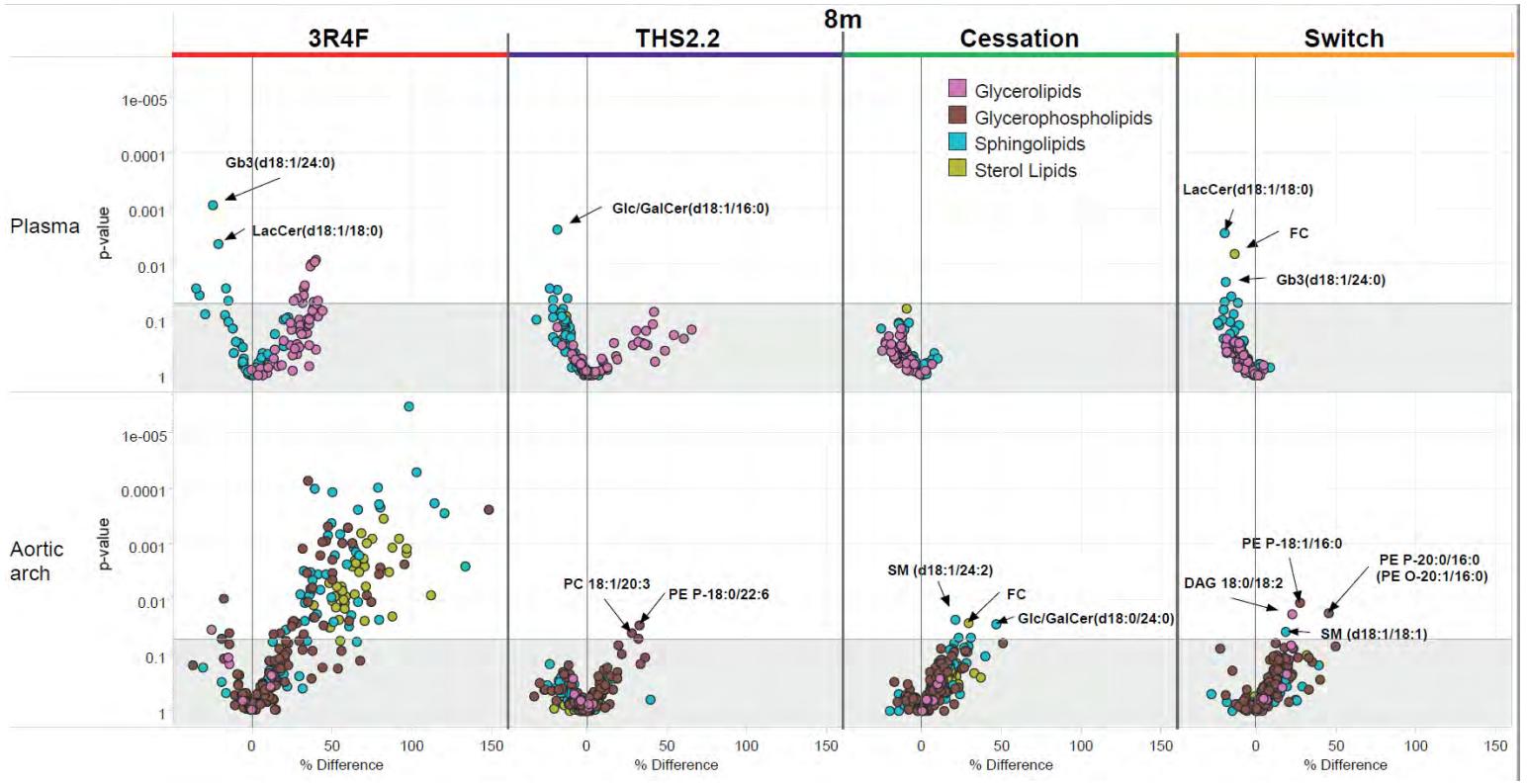
Lipidomics Profiling in Aorta and Plasma



8-month time point, lipid classes



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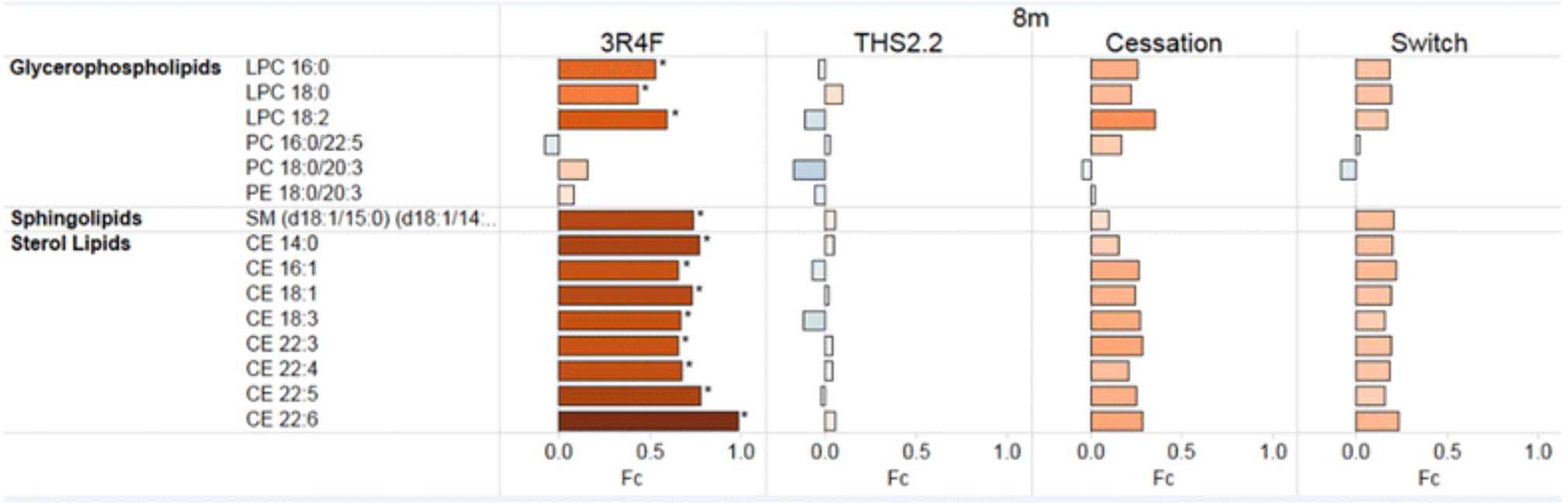
Aortic Arch Lipids from CS-Exposed ApoE$^{-/-}$ Mice and Human Plaque-Enriched Lipids

Aortic arch lipids from exposed ApoE$^{-/-}$ mice vs. sham controls share plaque-enriched lipids found in human carotid endarterectomies - reported by Stegemann et al.



"The lipid classes accounting for the major differences between control and diseased arteries were cholesteryl esters (CEs,) sphingomyelins (SMs), triacylglycerol, PC/lysoPC (lPCs), and phosphatidylcholines (PCs)"
Stegemann, C., et al. (2011)

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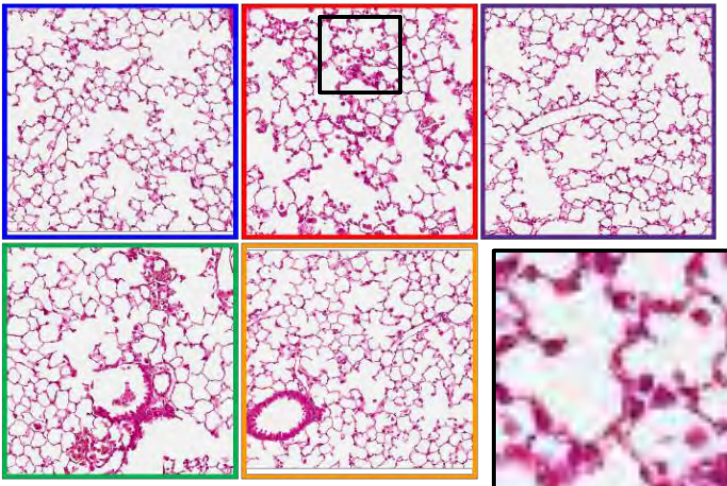
Stegemann, C., et al. (2011). "Comparative lipidomics profiling of human atherosclerotic plaques." Circulation: Cardiovascular Genetics: CIRCGENETICS. 110.959098.

Respiratory Tract Histology



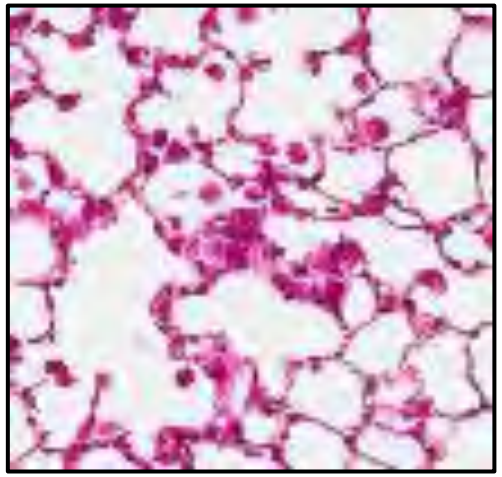
Sham

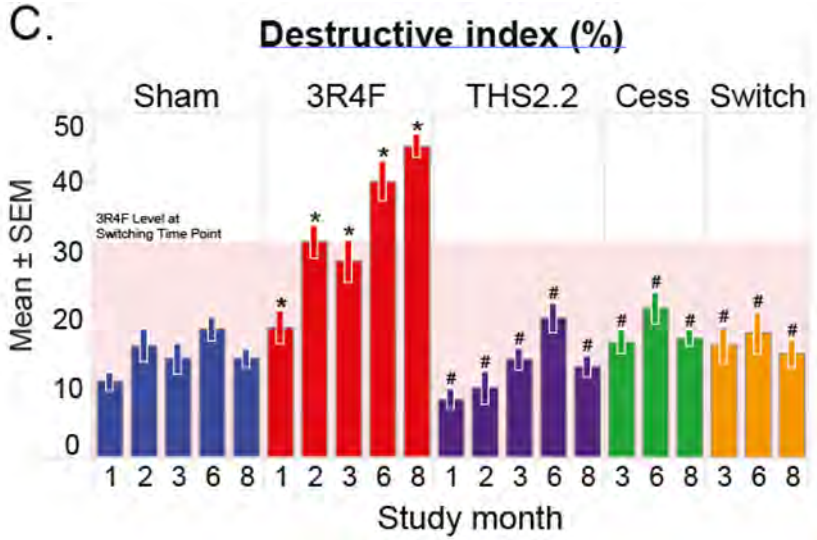
Reference cigarette

THS 2.2

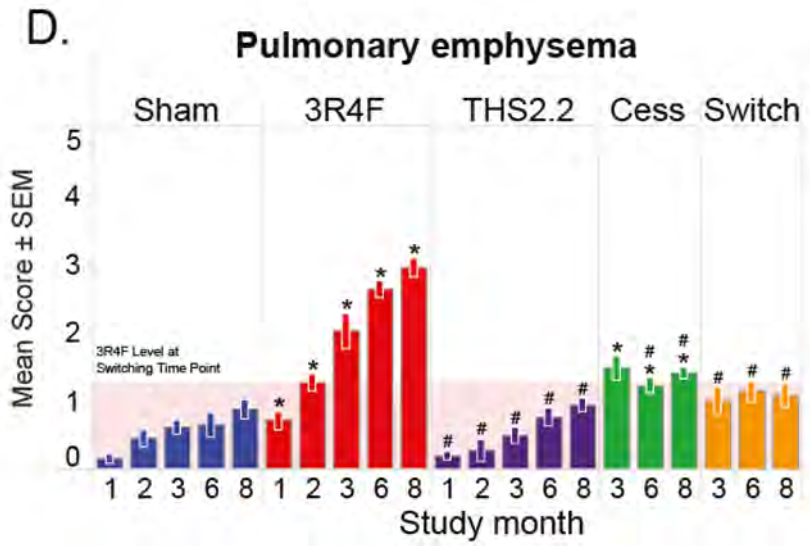
Cessation

Switch





➢ Morphometric anaylsis indicates CS-induced emphysema



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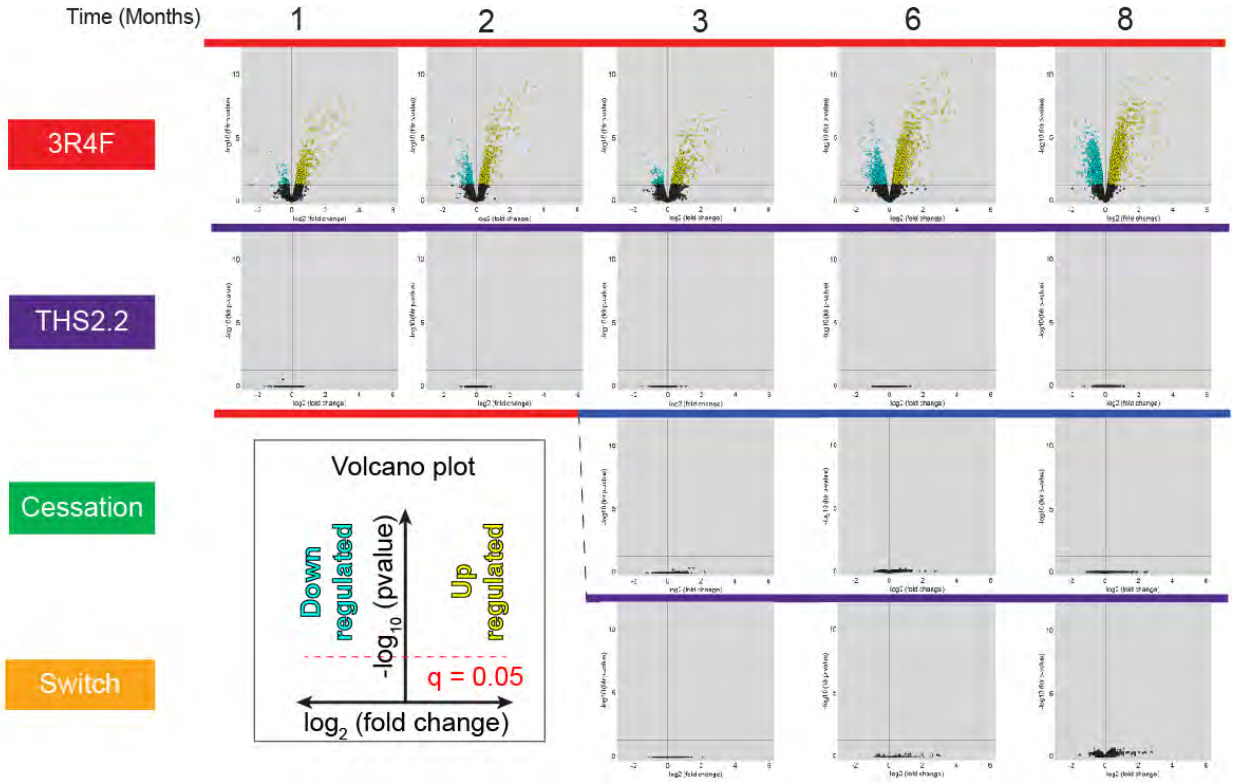
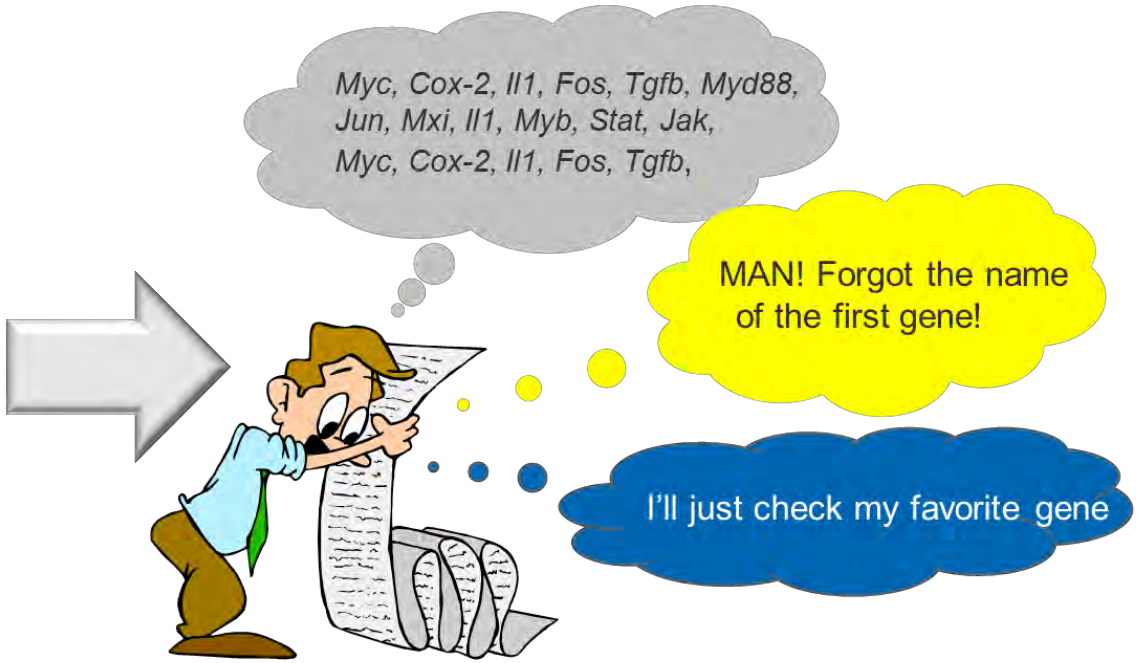
Differentially expressed genes (all vs. sham)



Biological interpretation



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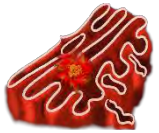
Mechanisms Impacted in the Lung

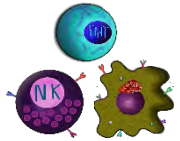
Cell Fate

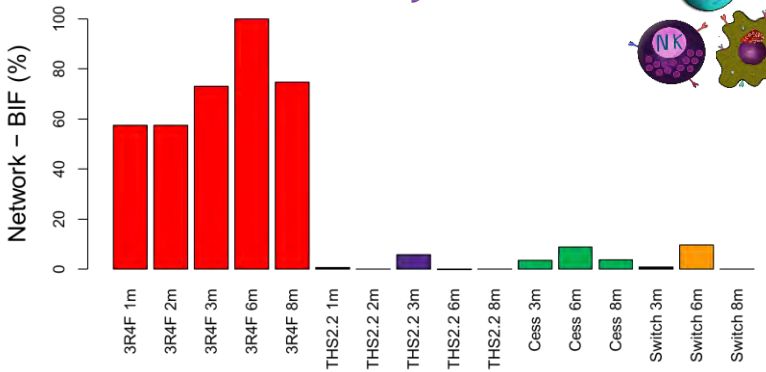
Cell Proliferation

Cell Stress

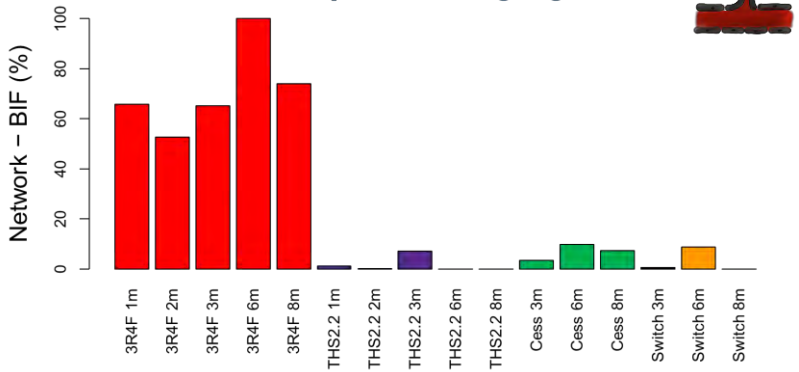


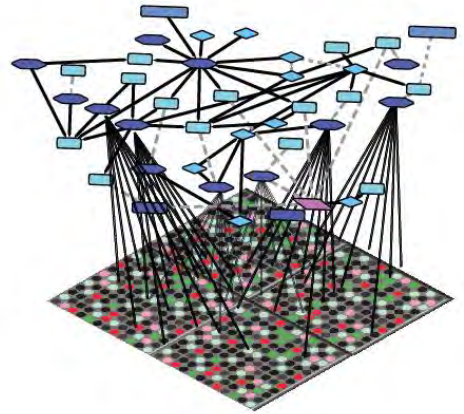
Inflammatory Processes





Tissue Repair & Angiogenesis





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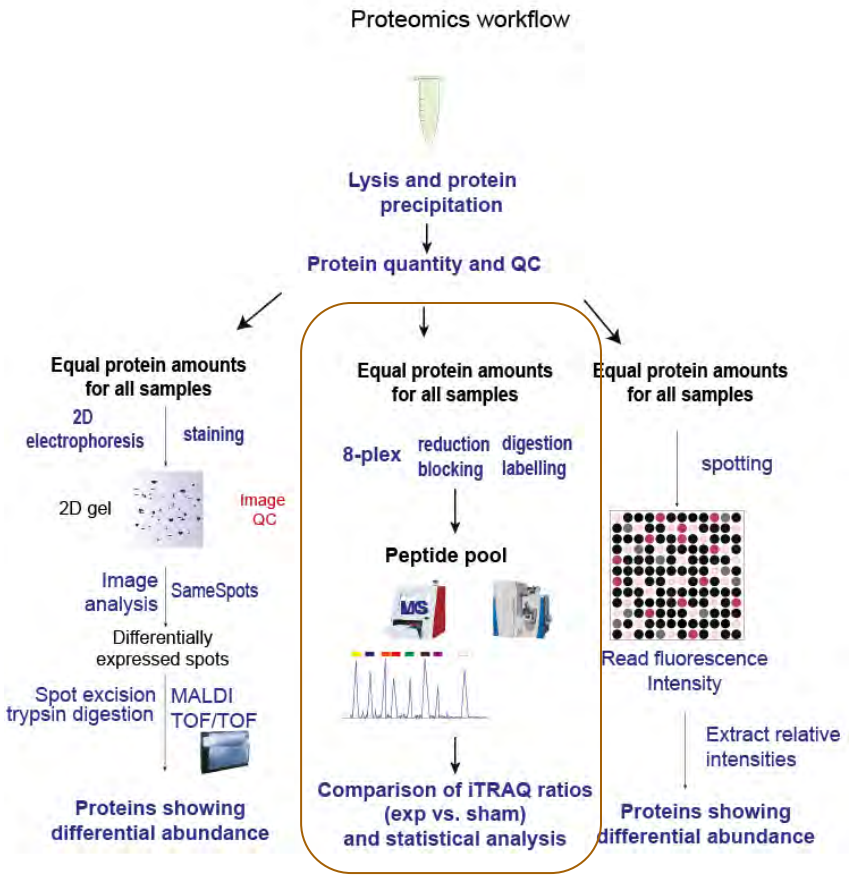
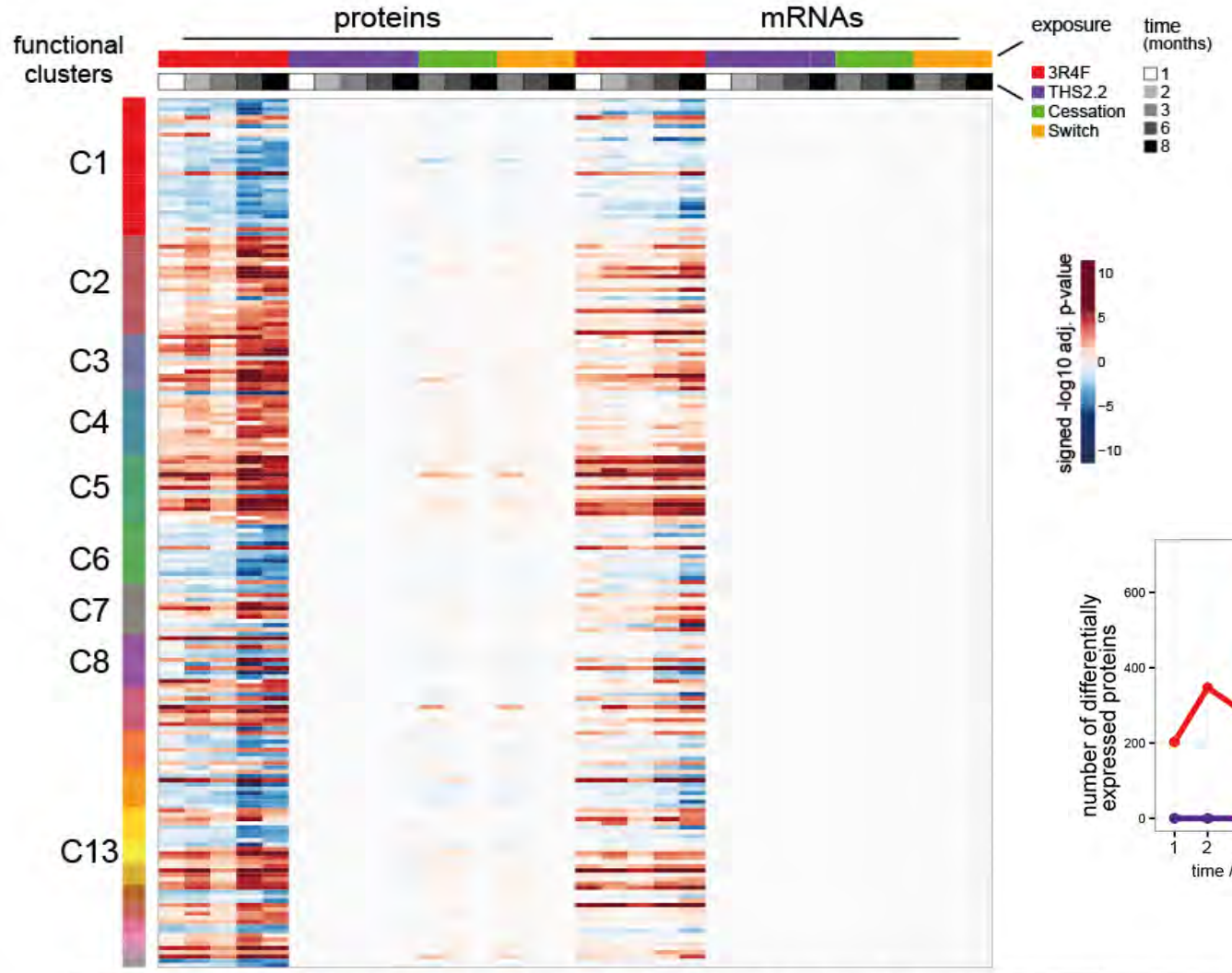
Phillips, B., et al. (2015). "An 8-month systems toxicology inhalation/cessation study in Apoe−/− mice to investigate cardiovascular and respiratory exposure effects of a candidate modified risk tobacco product, THS 2.2, compared with conventional cigarettes." Toxicological Sciences 149(2): 411-432.

Proteomics Profiling of the Lung



LC-MS-based

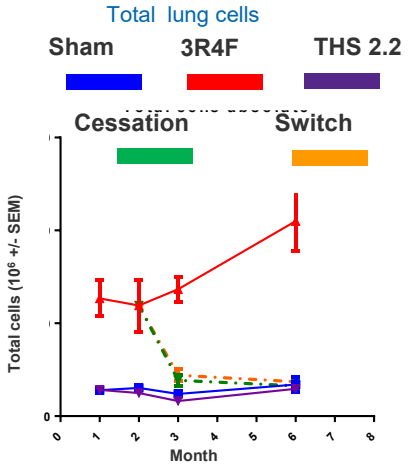


Phillips, B., et al. (2015). "An 8-month systems toxicology inhalation/cessation study in Apoe−/− mice to investigate cardiovascular and respiratory exposure effects of a candidate modified risk tobacco product, THS 2.2, compared with conventional cigarettes." Toxicological Sciences 149(2): 411-432.

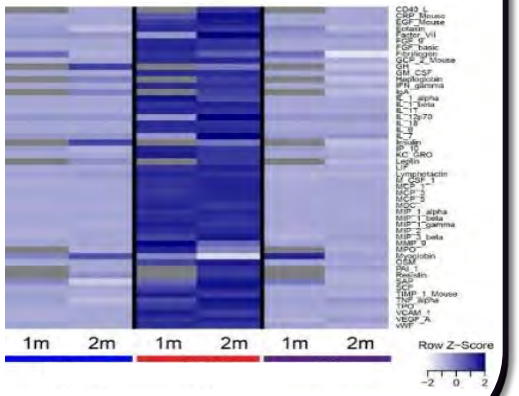
Systems Toxicology Approach to Investigate Cardiovascular and Pulmonary Disease for THS2.2

Lung Inflammation

Total lung cells

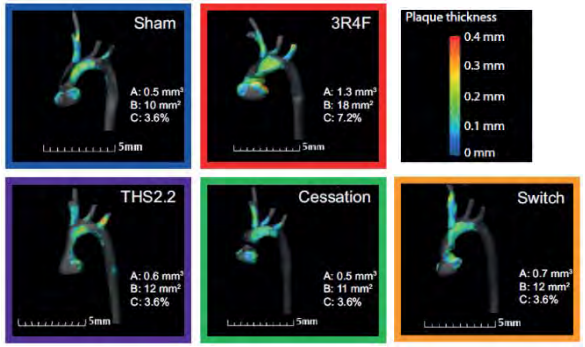


Mediators in BALF

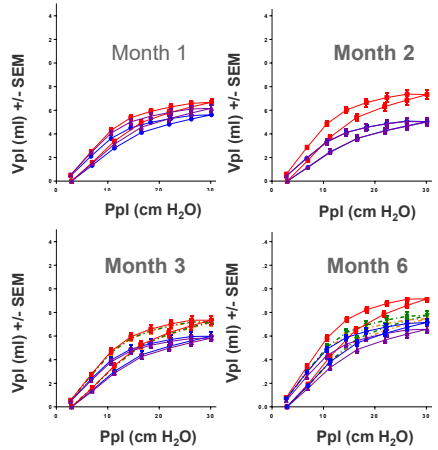


Cardiovascular

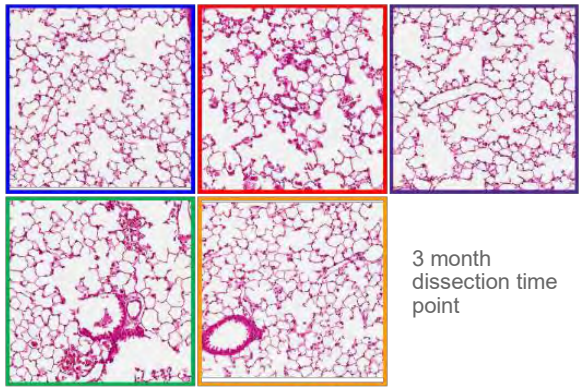
Micro CT



Lung function



Lung histopathology



3 month dissection time point

Biomarkers/Endpoints

Biomarkers/Endpoints		Summary of effect versus 3R4F		
		THS 2.2	Cessation	Switching to THS 2.2
Exposure markers	NNAL, 3-HPMA, SPMA, CEMA, COHb	↘	↘	↘
Cardio vascular disease	Rate of atherosclerotic plaque growth	↘	↘	↘
	Blood lipidomics (including 11-DTX-B2 and isoprostanes)	↘	↘	↘
	Aorta lipidomics	↘	↘	↘
Respiratory disease	Lung function measured using a FlexiVent system	↘	↘	↘
	Histopathological evaluation of the respiratory nasal epithelium	↘	↘	↘
	Histopathological evaluation of the lung tissue	↘	↘	↘
	Inflammatory mediators and cells in the bronchoalveolar lavage fluid (BALF)	↘	↘	↘
	Lung lipidomics	↘	↘	↘
	Whole transcriptome analysis of the Respiratory Nasal Epithelium: Perturbation of xenobiotic metabolism, inflammation, hypoxia, apoptosis, cell proliferation.	↘	↘	↘
	Whole transcriptome analysis of the lung tissue: Perturbation of xenobiotic metabolism, inflammation, hypoxia, apoptosis, cell proliferation.	↘	↘	↘

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Publications

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

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

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

Taylor & Francis

Inhalation Toxicology
International Forum for Respiratory Research

ISSN: 0895-8378 (Print) 1091-7691 (Online) Journal homepage: http://www.tandfonline.com/loi/iiht20

Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver in Apoe$^{-/-}$ mice – a systems toxicology analysis

Giuseppe Lo Sasso, Bjoern Titz, Catherine Nury, Stéphanie Boué, Blaine Phillips, Vincenzo Belcastro, Thomas Schneider, Sophie Dijon, Karine Baumer, Daruisz Peric, Remi Dulize, Ashraf Elamin, Emmanuel Guedj, Ansgar Buettner, Patrice Leroy, Samuel Kleinhans, Gregory Vuillaume, Emilija Veljkovic, Nikolai V. Ivanov, Florian Martin, Patrick Vanscheeuwijck, Manuel C. Peitsch & Julia Hoeng

Food and Chemical Toxicology 101 (2017) 157–167

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Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Aerosol from Tobacco Heating System 2.2 has reduced impact on mouse heart gene expression compared with cigarette smoke

Justyna Szostak [a, *], Stéphanie Boué [a], Marja Talikka [a], Emmanuel Guedj [a], Florian Martin [a], Blaine Phillips [b], Nikolai V. Ivanov [a], Manuel C. Peitsch [a], Julia Hoeng [a]

[a] Philip Morris International R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland
[b] Philip Morris International Research Laboratories Pte Ltd, Science Park II, Singapore

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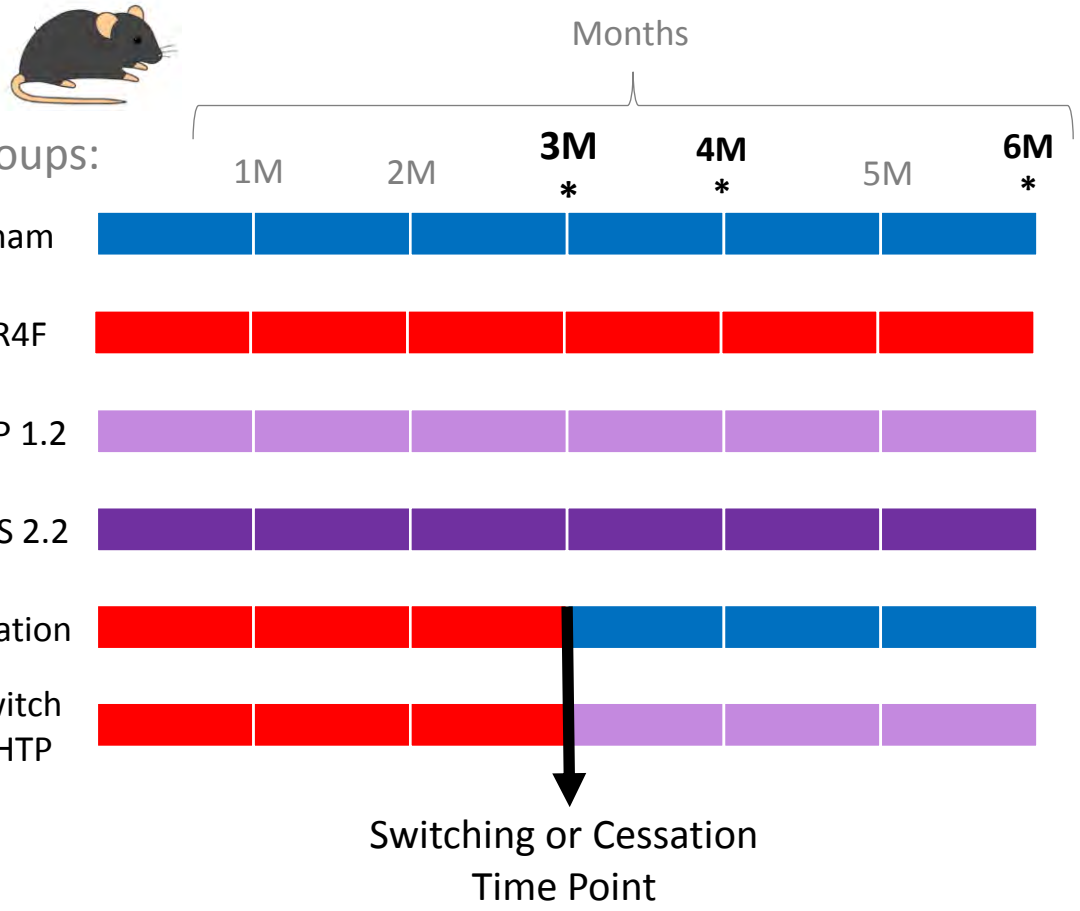
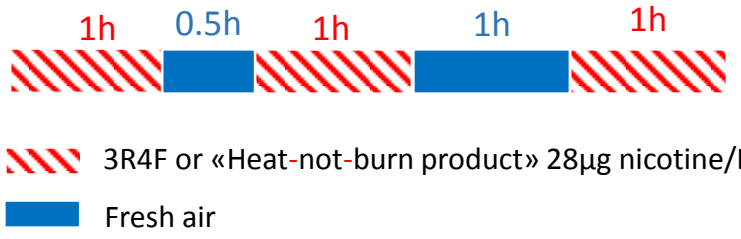
Study Design - CHTP1.2-ApoE$^{-/-}$



Exposure:



Objective:

The objective of the study was to investigate the impact of CS or aerosol from two potential MRTPs, CHTP 1.2 and THS 2.2, on the cardiovascular and respiratory system.

ApoE$^{-/-}$ mice were exposed to CS, or to the aerosol from MRTPs (THS 2.2 or CHTP 1.2 over a 6-month period.

The effects of cessation or switching to CHTP 1.2 aerosol after 3 months of CS exposure were also investigated.

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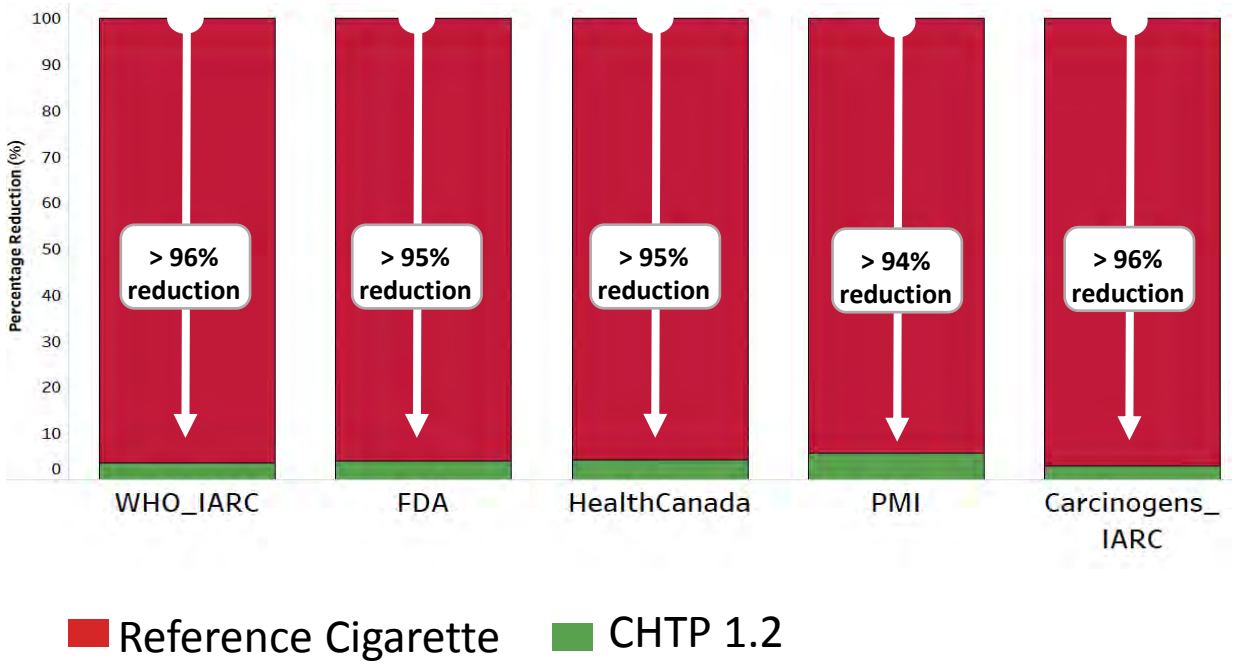
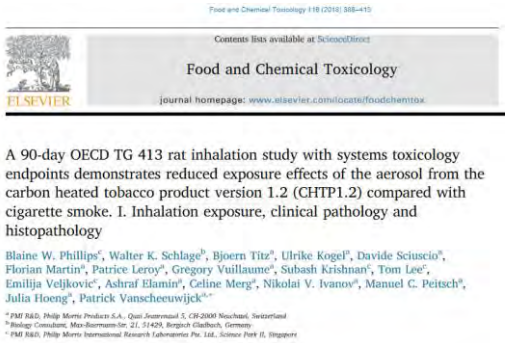
Average reductions in formation of HPHCs for CHTP 1.2 compared with levels measured in smoke from the 3R4F reference cigarette*



*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, nicotine-free dry particulate matter, water, glycerin, total particulate matter. The PMI 58 list includes the FDA 18 and the 15 carcinogens of the IARC Groups 1.


Contents lists available at ScienceDirect

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A 90-day OECD TG 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of the aerosol from the carbon heated tobacco product version 1.2 (CHTP1.2) compared with cigarette smoke. I. Inhalation exposure, clinical pathology and histopathology

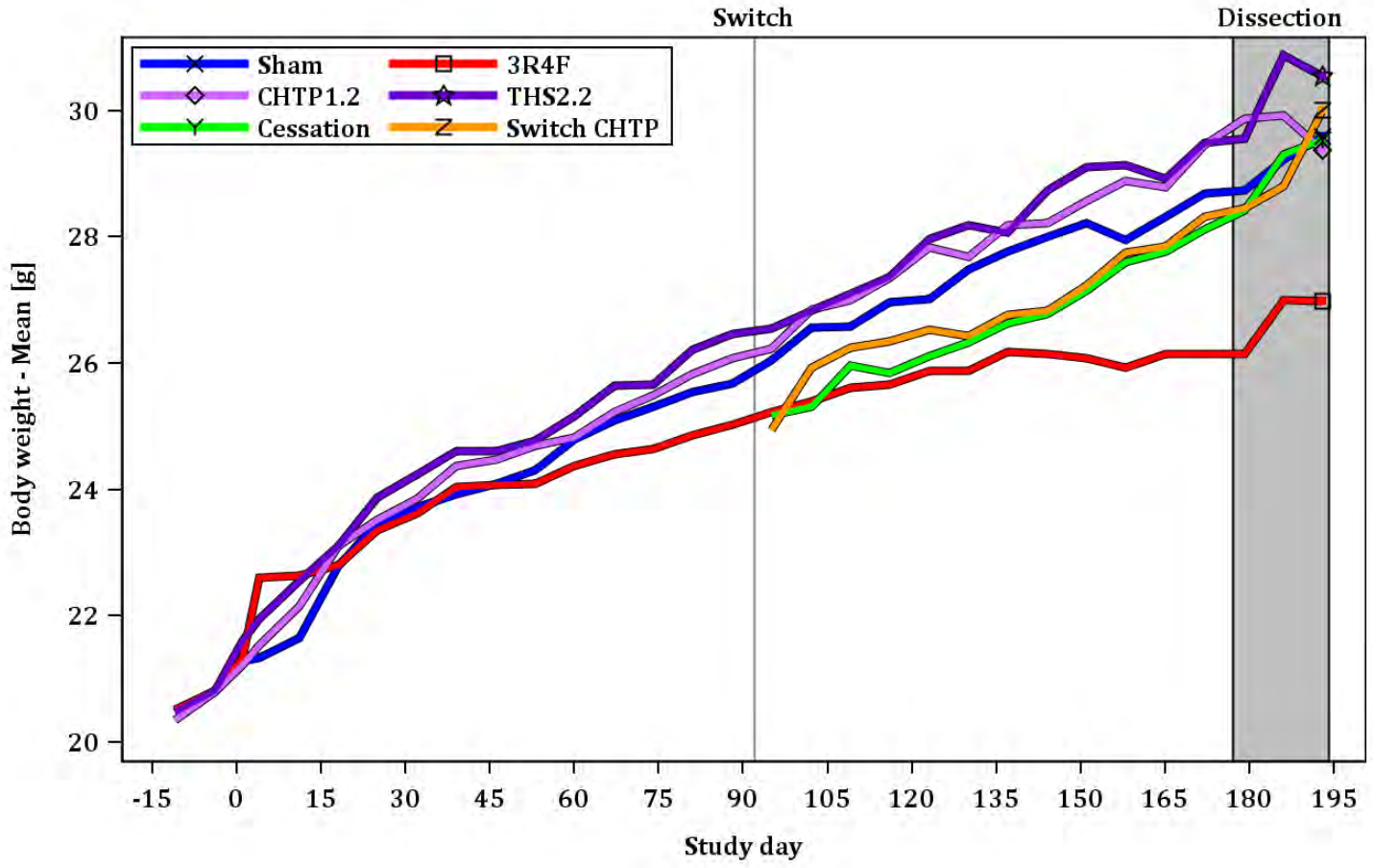
CHTP is a single-use, disposable tobacco product that resembles a cigarette and that is to be used in a similar manner as a cigarette.

CHTP uses a fast-lighting carbon heat source to heat a tobacco plug in a specially designed stick to produce an aerosol that contains nicotine and tobacco flavor.

During use, the tobacco in the tobacco stick does not exceed a well-defined temperature threshold, which prevents combustion of the tobacco and consequently significantly limits the generation and delivery of harmful smoke constituents into the aerosol.

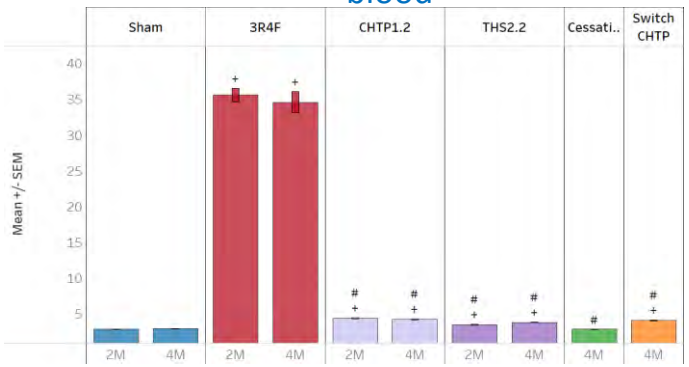
Phillips BW et al. A 90-day OECD TG 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of the aerosol from the carbon heated tobacco product version 1.2 (CHTP1.2) compared with cigarette smoke. I. Inhalation exposure, clinical pathology and histopathology. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2018.

Bodyweight Curves



In Blood

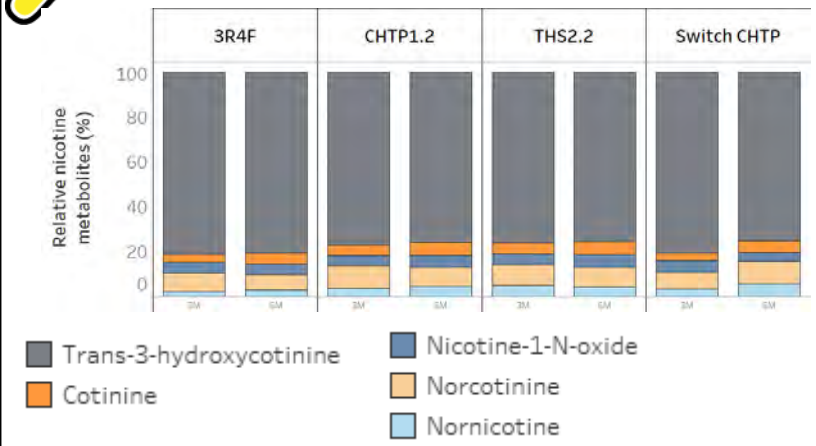
Biomarker of CS exposure COHb (carboxyhemoglobin concentration) measured in blood



\+ $p < 0.05$ significant versus sham
\# $p < 0.05$ significant versus 3R4F

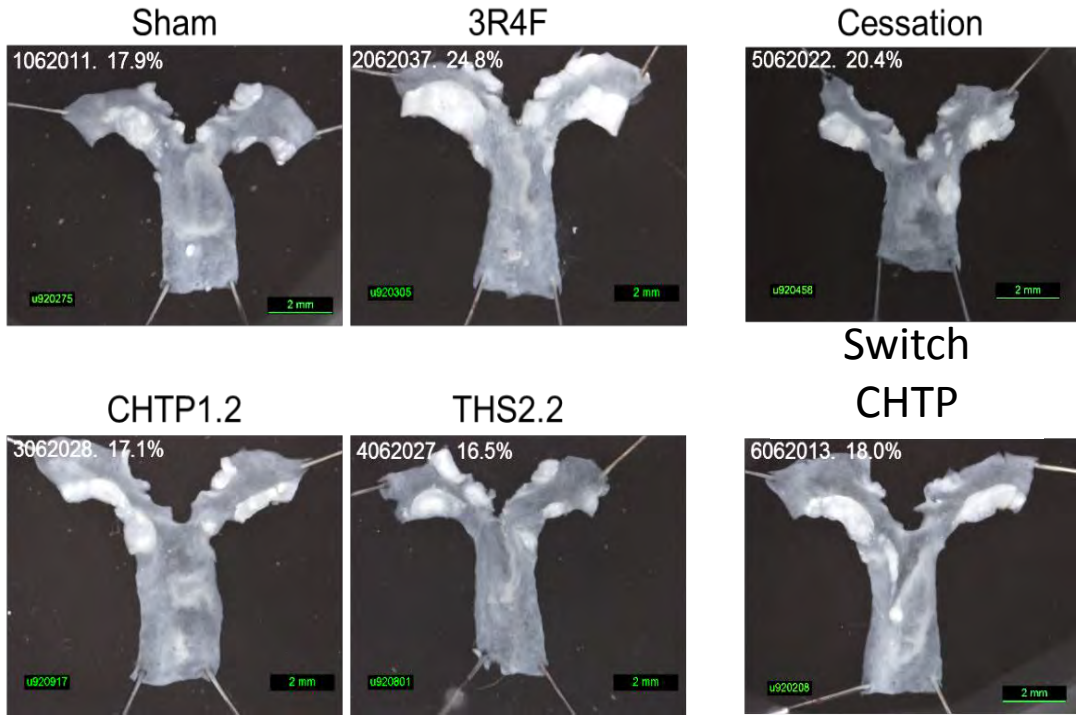
In Urine

Nicotine metabolites in exposed groups

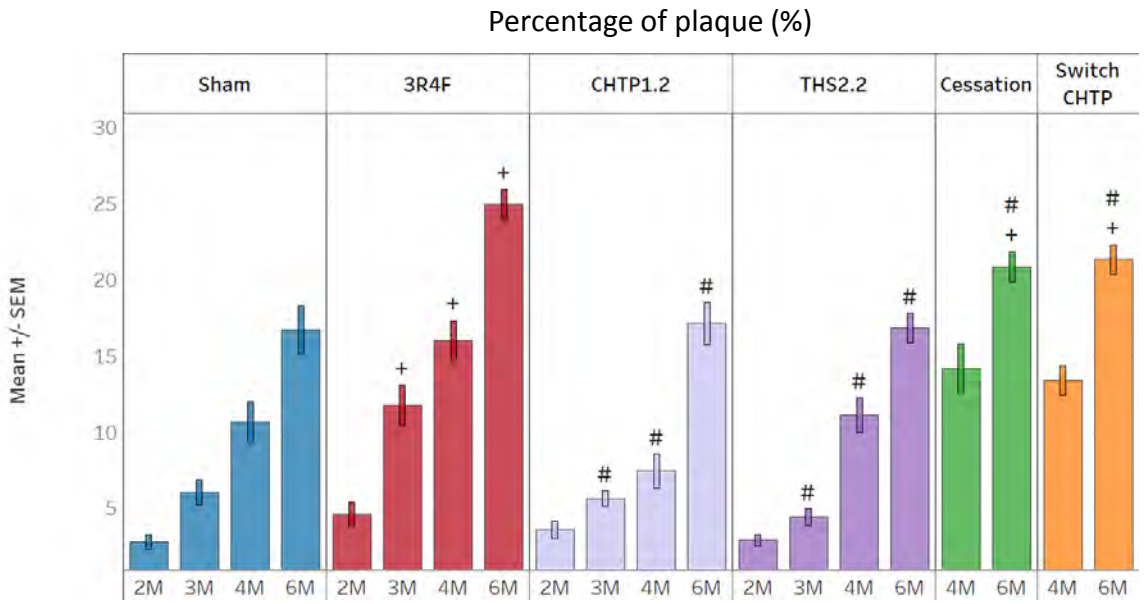


Disease Endpoint - Aortic Plaque Progression in ApoE$^{-/-}$ Mice Study 2

Aortic arch plaque area measurements



Planimetry pictures representing plaque area in aortic arch



\+ $p < 0.05$ significant versus sham
\# $p < 0.05$ significant versus 3R4F

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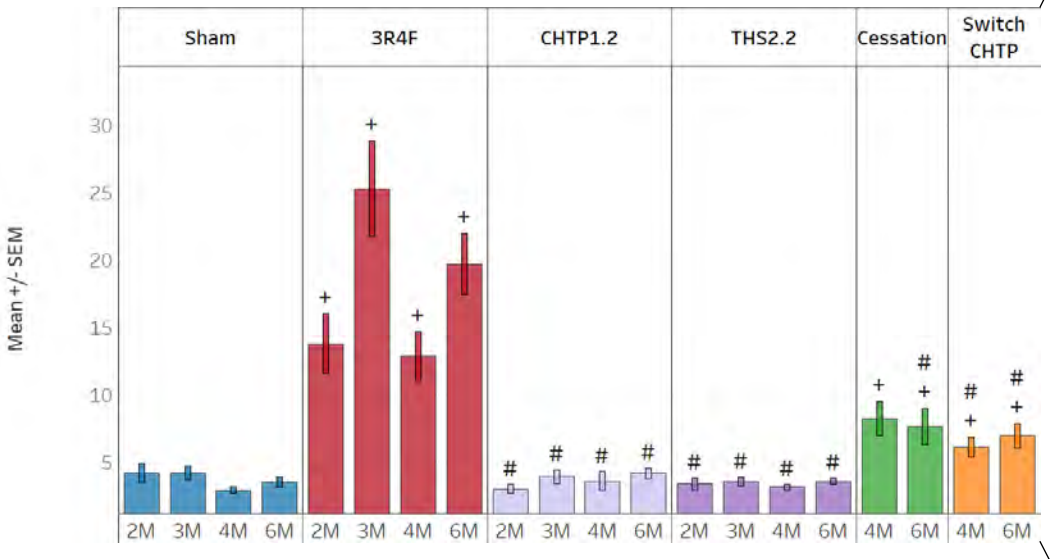
Free Lung Cells in BALF in ApoE$^{-/-}$ Mice Study 2

Inflammatory cells in BALF

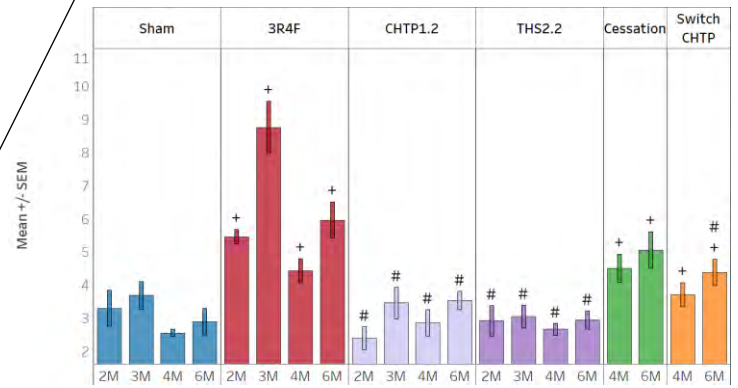
Total cells (x10^5)



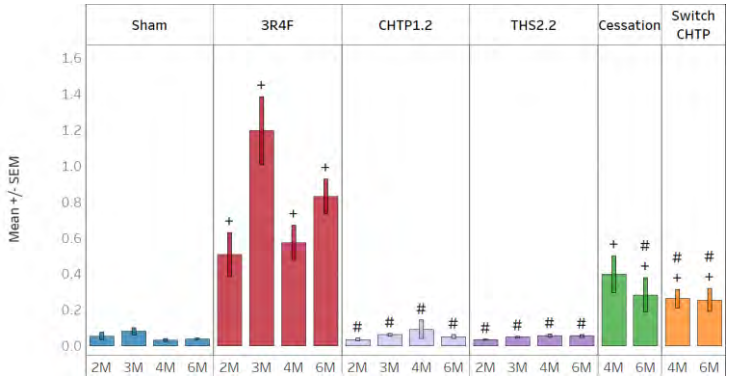
+ $p < 0.05$ significant versus sham
# $p < 0.05$ significant versus 3R4F

Differential counts

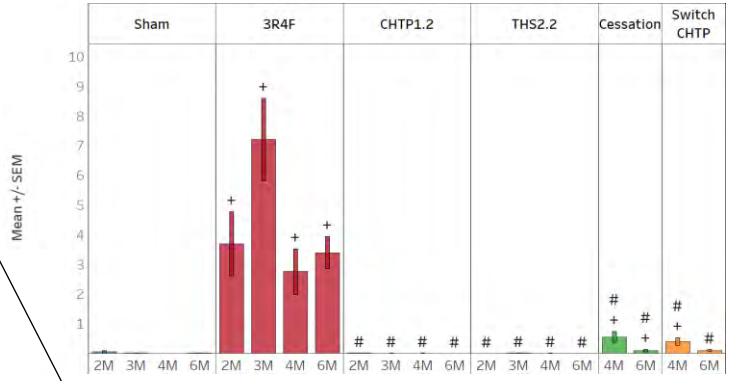
Alveolar macrophages (x10^5)



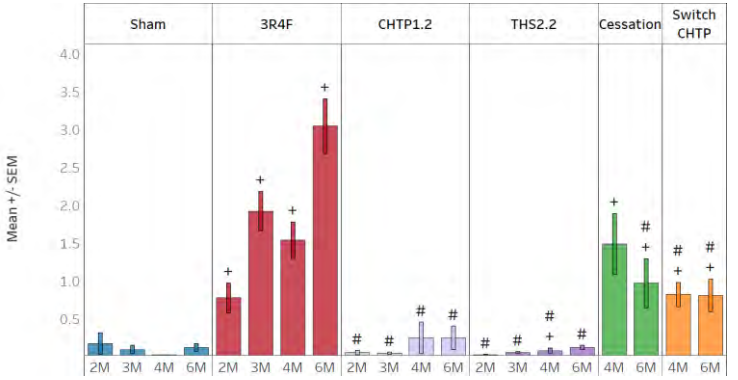
Alveolar dendritic cells (x10^5)



Neutrophils (x10^5)

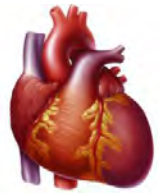


Lymphocytes (x10^5)



Heart (left ventricle) Transcriptomics in ApoE$^{-/-}$ mice study 2

Left ventricle

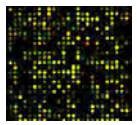


RNA extraction

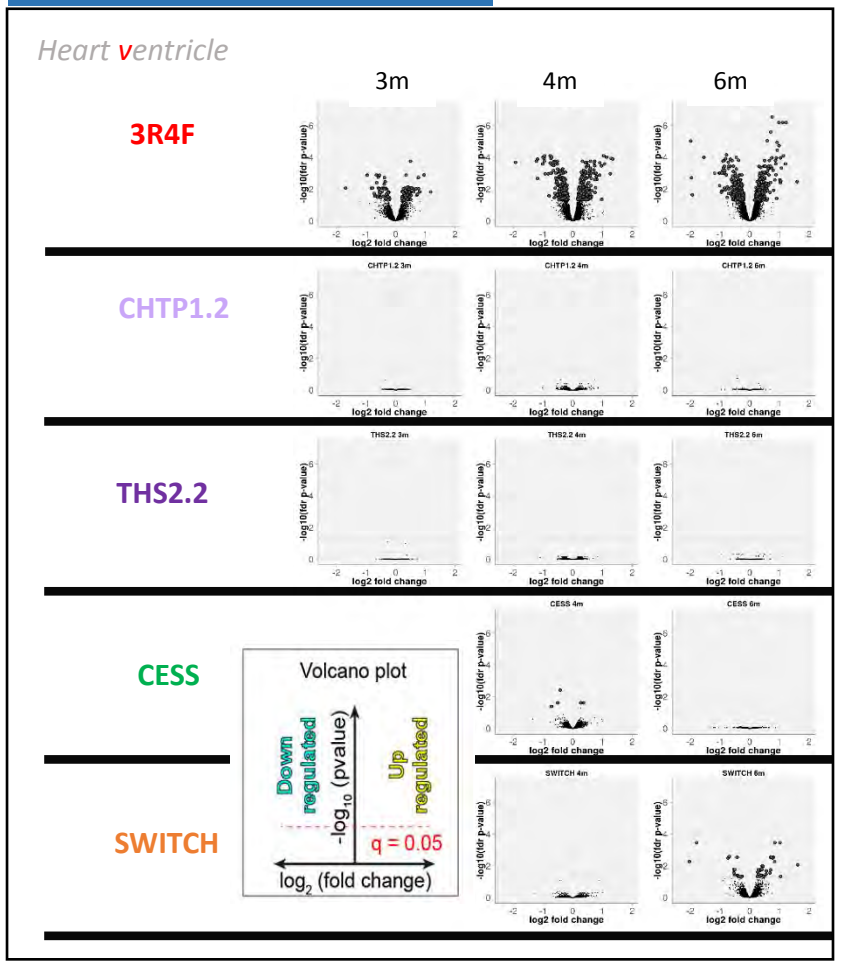
cDNA transformation+ labelling

Hybridized

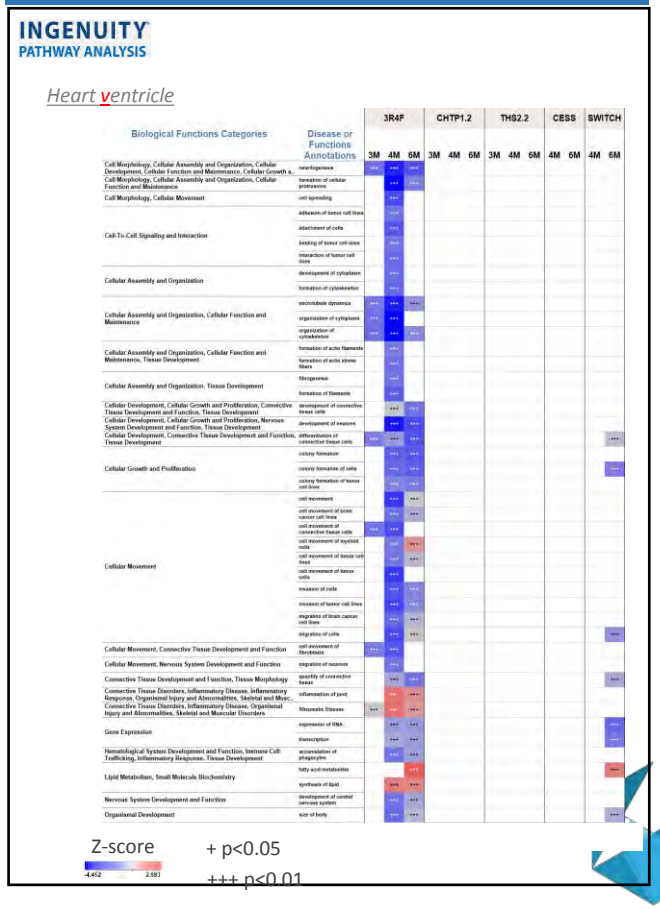
Scan



TRANSCRIPTOMICS



TRANSCRIPTOMICS RESULTS



- Muscle structure and function
- Cellular assembly and organization
- Inflammatory response

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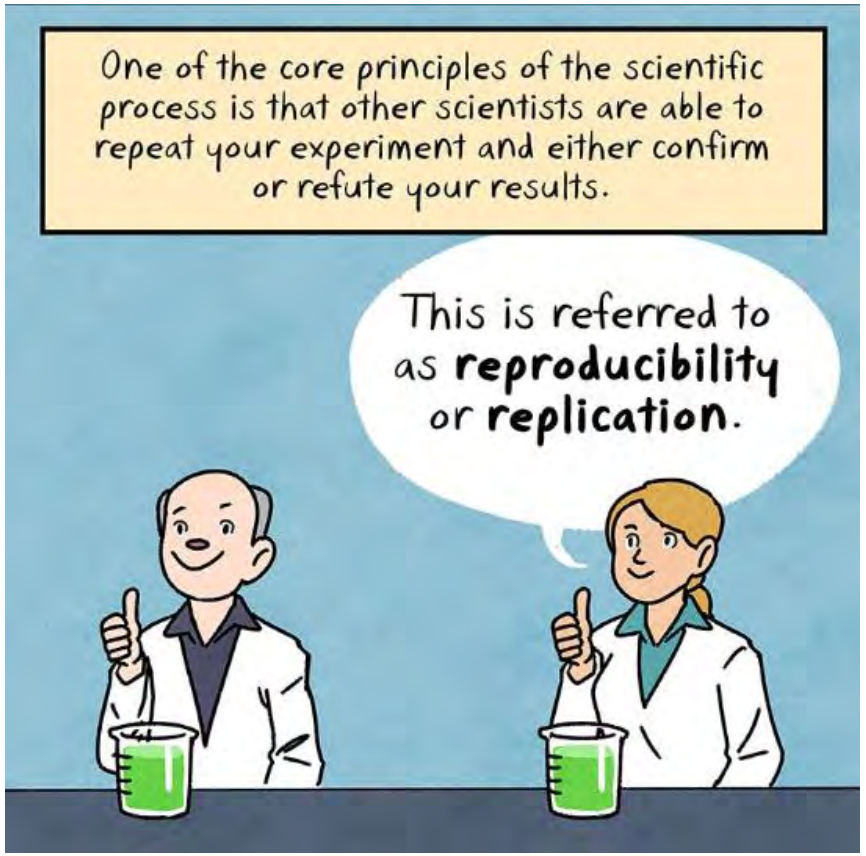
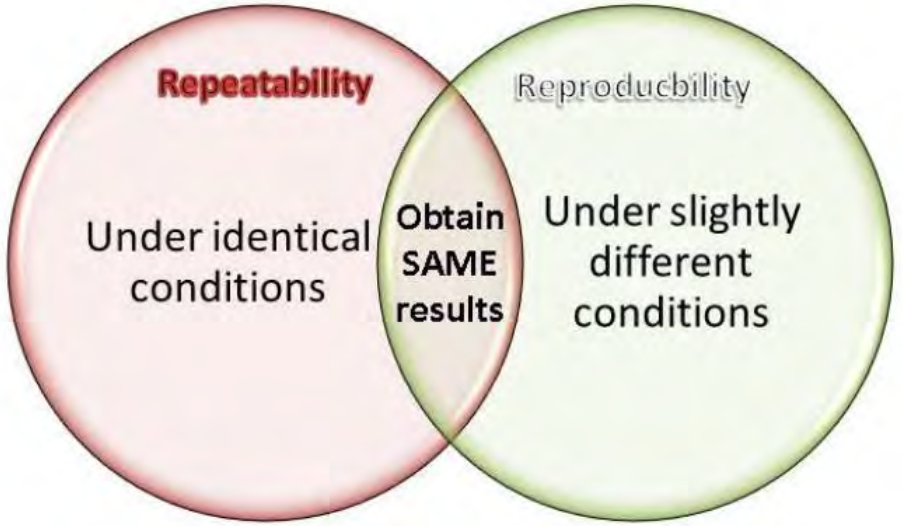


Are the results for THS 2.2 reproducible in two independent studies?



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Biomarkers of exposure in ApoE$^{-/-}$ Study 1 versus Study 2 for 3R4F and THS 2.2

In Urine

3R4F Mean Relative to sham

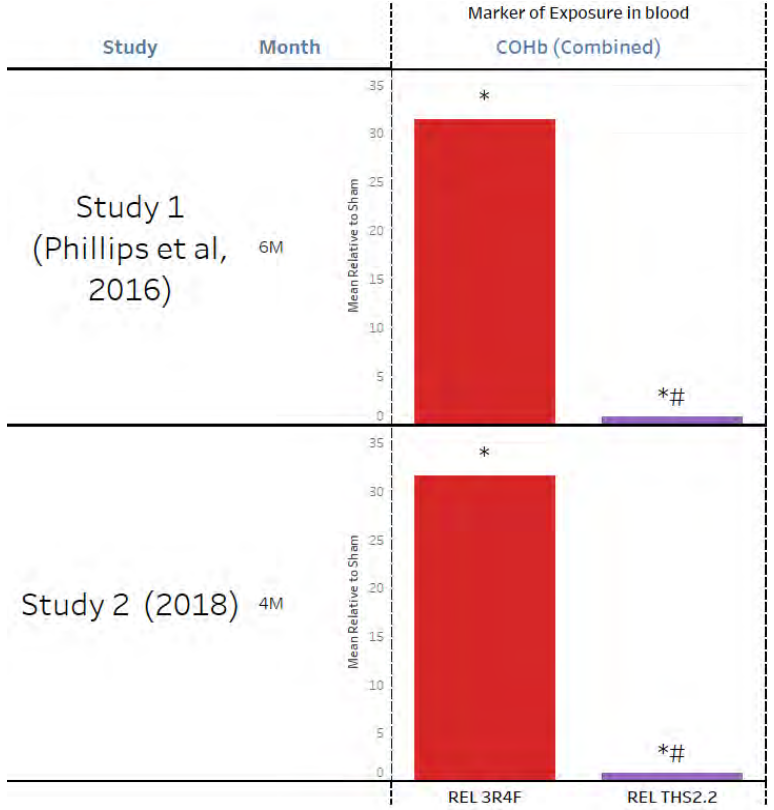
THS 2.2 Mean Relative to sham

* $p < 0.05$ significant versus sham

\# $p < 0.05$ significant versus 3R4F

In Blood

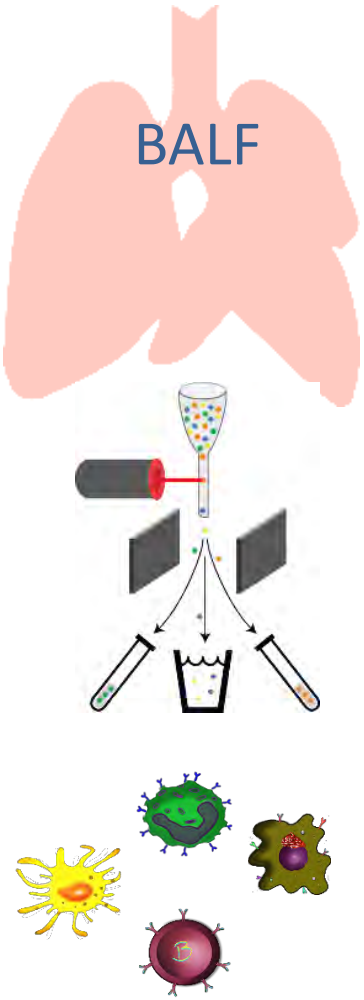
Biomarker of CS exposure COHb (carboxyhemoglobin concentration) measured in blood



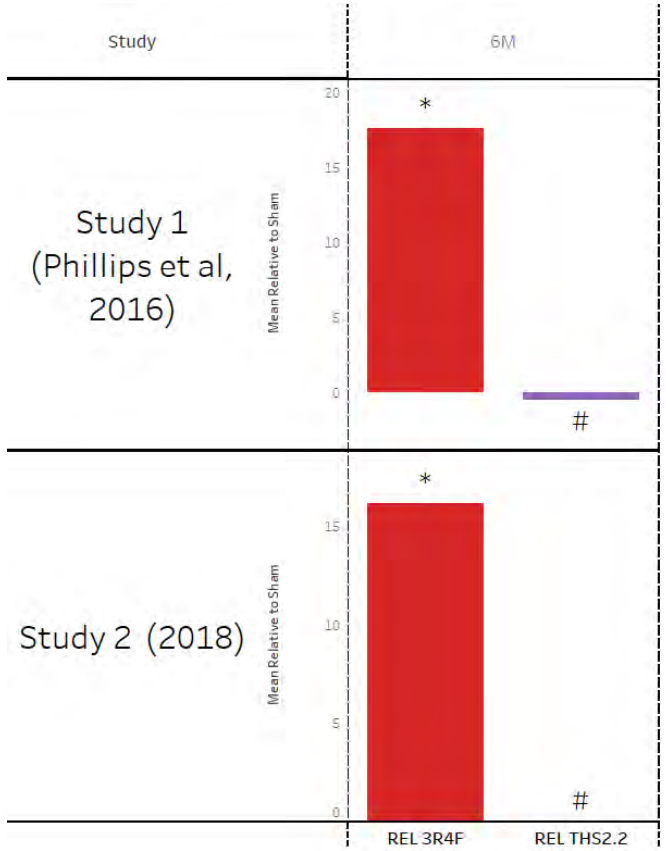
* $p < 0.05$ significant versus sham

\# $p < 0.05$ significant versus 3R4F

Inflammatory cells in ApoE$^{-/-}$ Study 1 versus Study 2 for 3R4F and THS 2.2

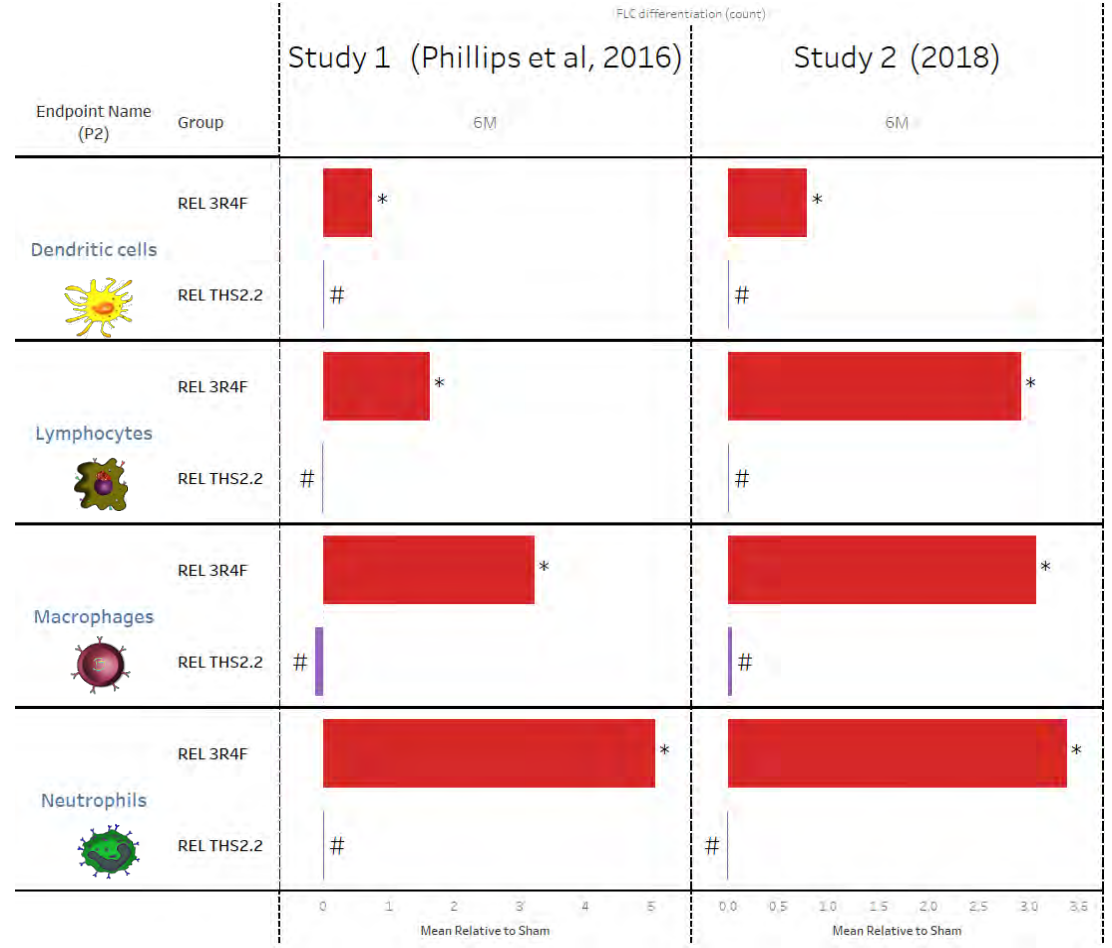


Total Cells Count



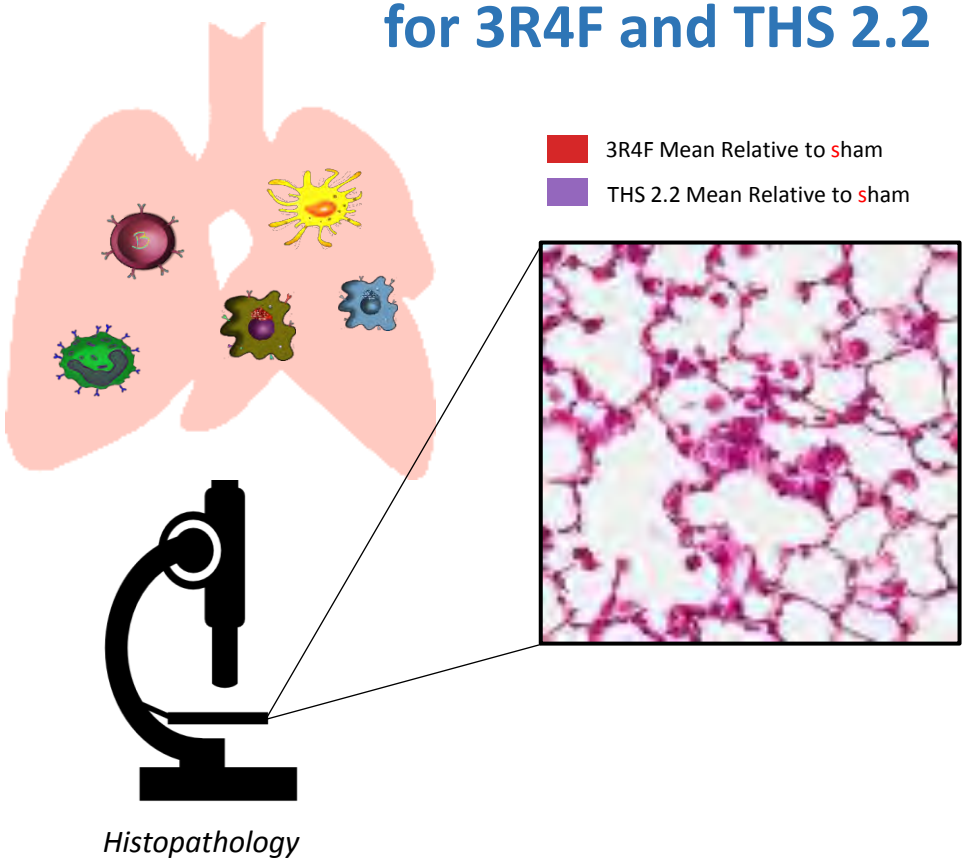
3R4F Mean Relative to sham
THS 2.2 Mean Relative to sham

Differential counts

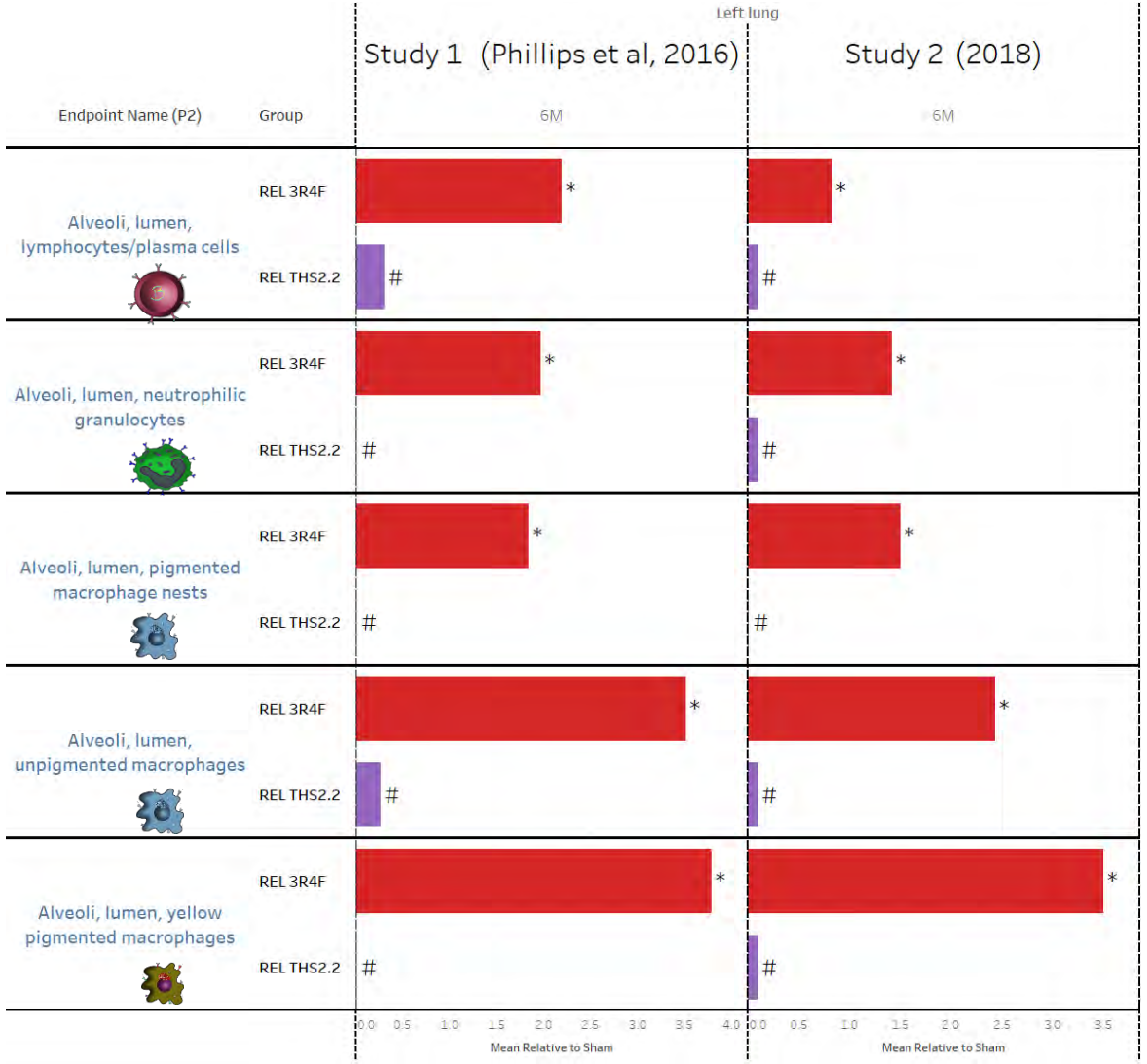


* $p < 0.05$ significant versus sham
\# $p < 0.05$ significant versus 3R4F

Inflammatory cells in lung tissue in ApoE$^{-/-}$ Study 1 versus Study 2 for 3R4F and THS 2.2



Histopathology

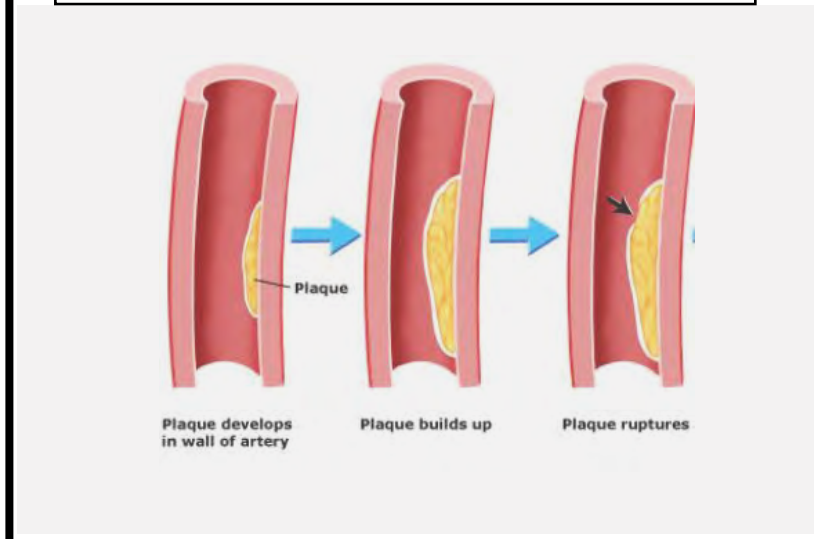


* $p < 0.05$ significant versus sham
\# $p < 0.05$ significant versus 3R4F

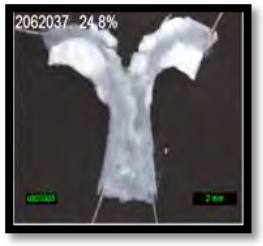
Disease Endpoint - Aortic Plaque Comparison for 3R4F and THS 2.2 Across Two ApoE$^{-/-}$ Studies

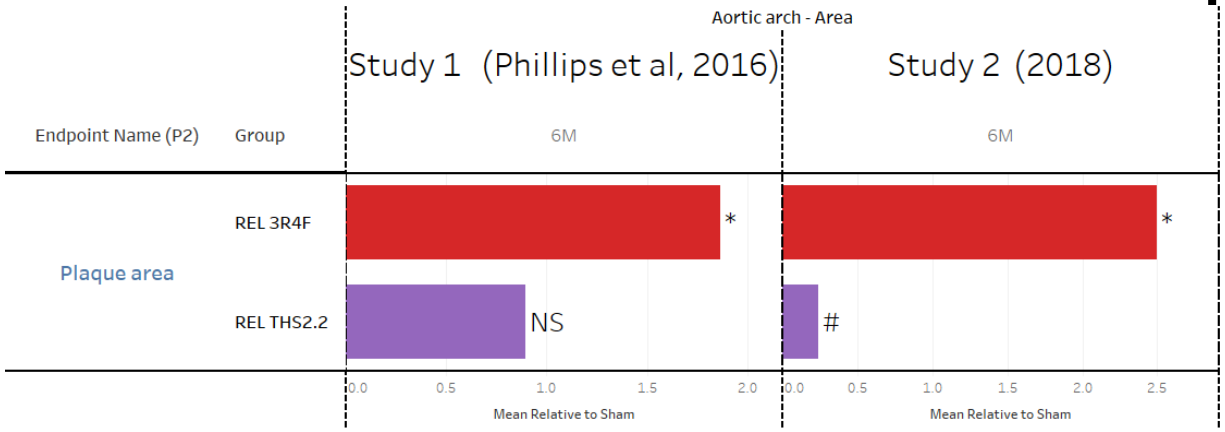
Atherosclerotic plaque progression

Plaque volume
Plaque area



Aortic arch plaque area measurements (planimetry)





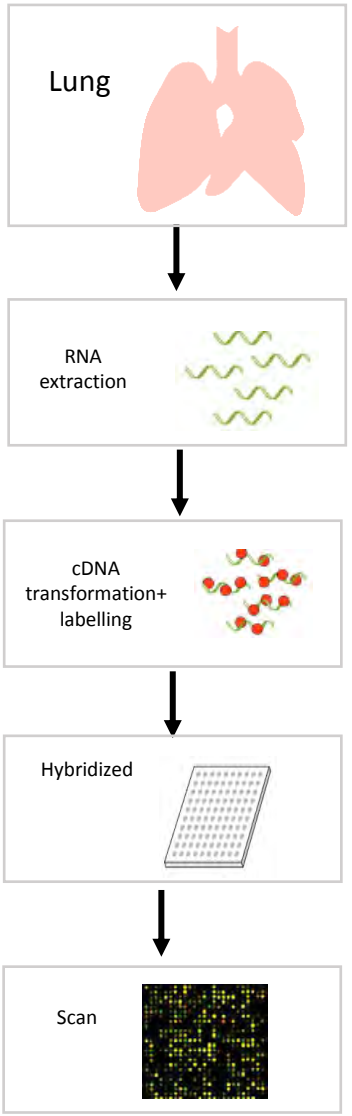
3R4F Mean Relative to sham

THS 2.2 Mean Relative to sham

* $p < 0.05$ significant versus sham

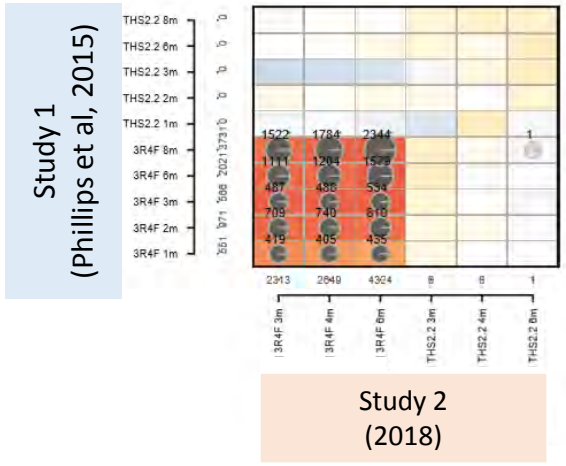
\# $p < 0.05$ significant versus 3R4F

Molecular Endpoint Comparison for 3R4F and THS 2.2 Across Two ApoE$^{-/-}$ Studies

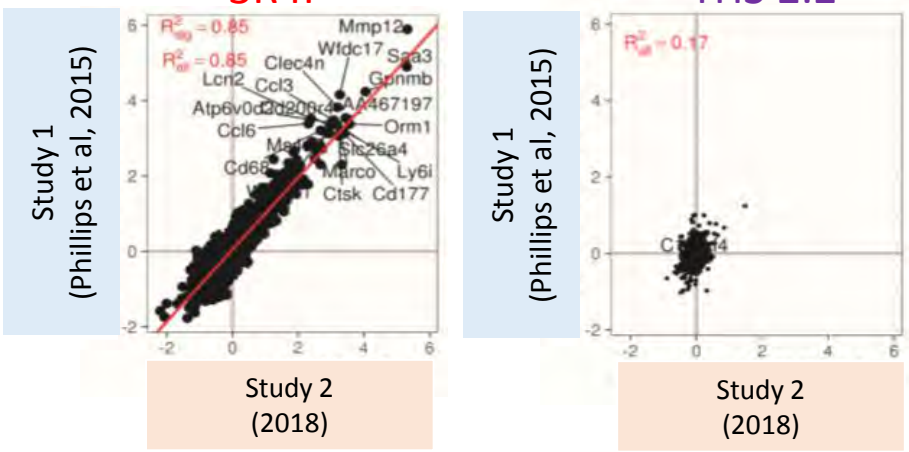


TRANSCRIPTOMICS

Number of differently expressed genes Study 1 versus Study 2 for 3R4F and THS 2.2

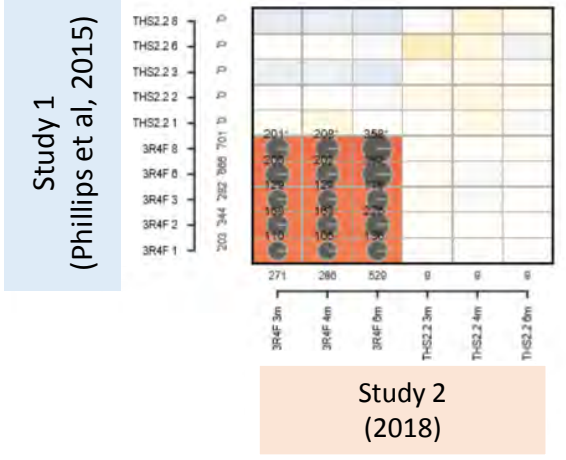


Study 1 versus Study 2 for 3R4F and THS 2.2 correlation at 6 months

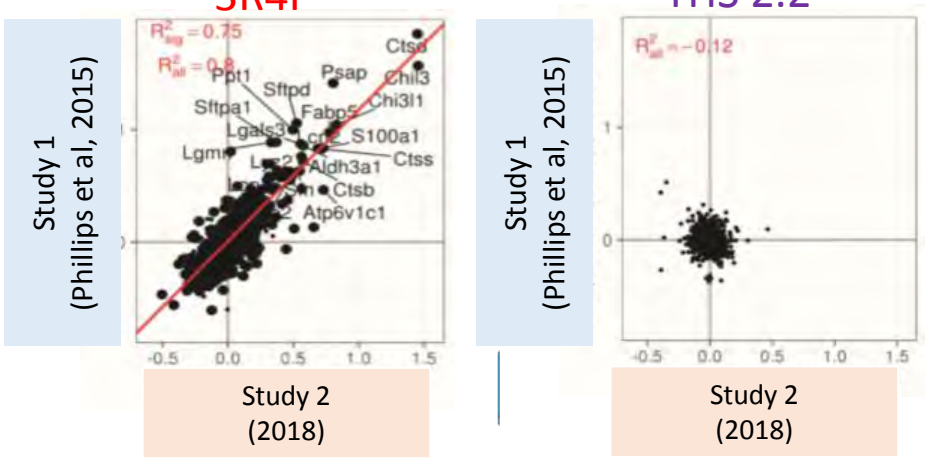


PROTEOMICS

Number of differently expressed genes Study 1 versus Study 2 for 3R4F and THS 2.2

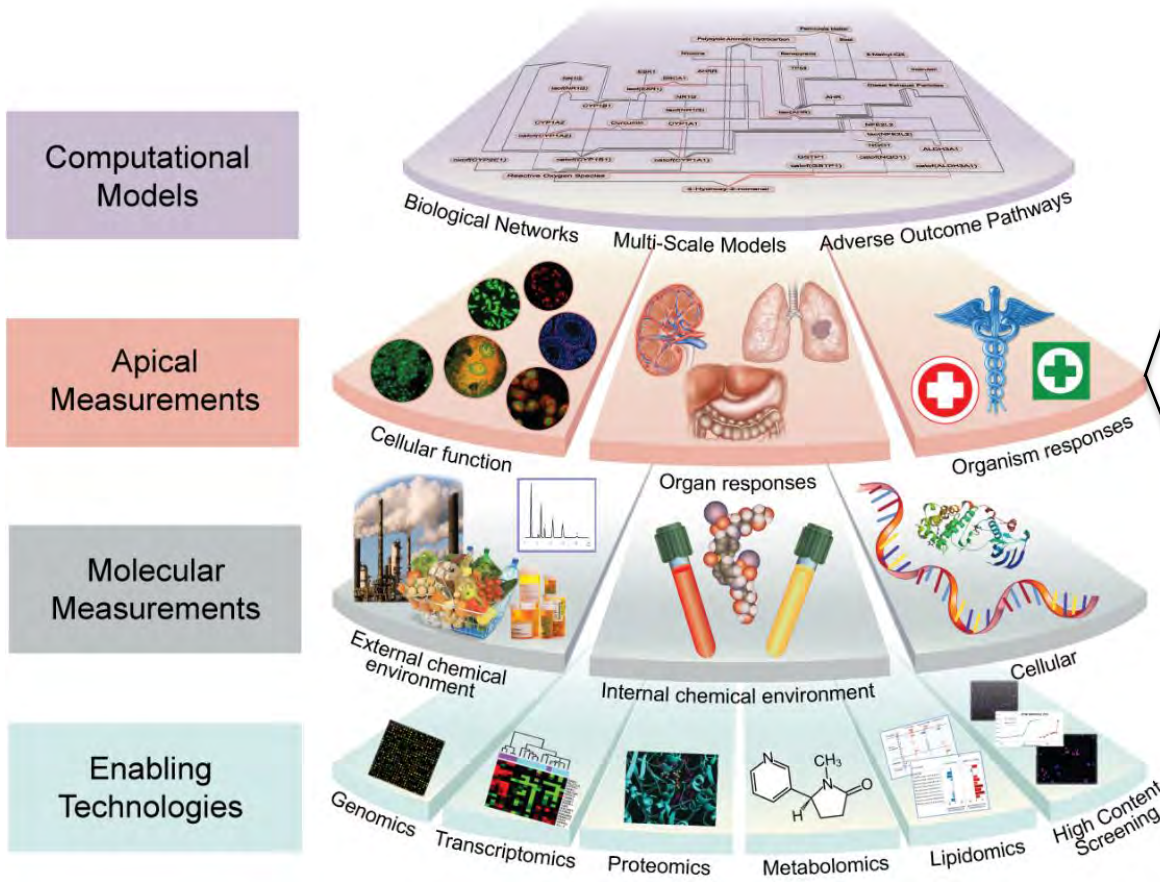


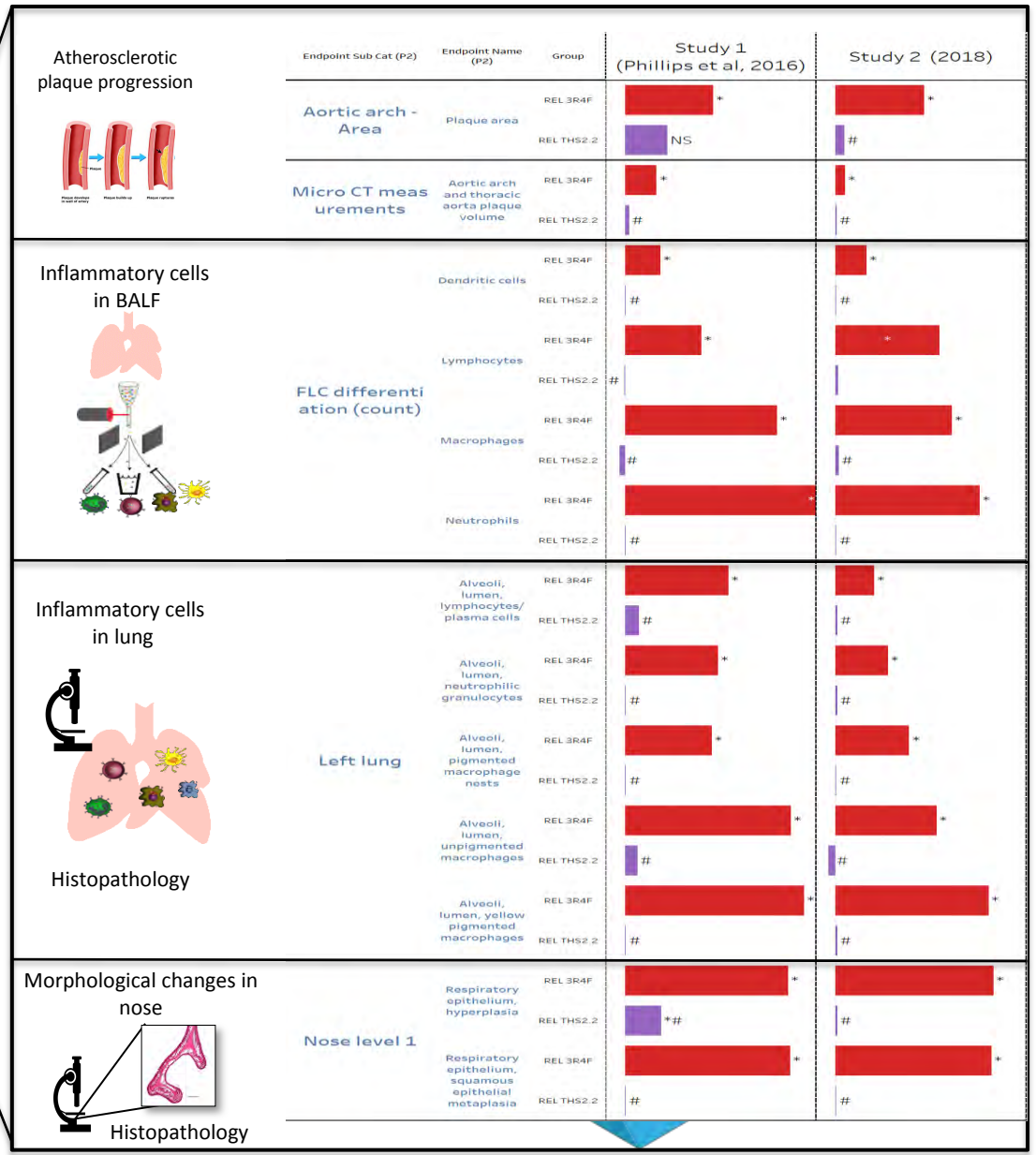
Study 1 versus Study 2 for 3R4F and THS 2.2 correlation at 6 months



Systems Toxicology Approach to Investigate Cardiovascular and Pulmonary Disease

Comparison for 3R4F impact and THS 2.2 across two ApoE$^{-/-}$ studies





* $p < 0.05$ significant versus sham
\# $p < 0.05$ significant versus 3R4F

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

Switching Study in an Animal Model of Disease - Summary and Conclusions

- The ApoE$^{-/-}$ mouse model is suitable for studying cardiovascular disease and COPD
- Cigarette smoke exposure accelerated the development of atherosclerotic plaque and emphysema.
- Continuous exposure to aerosol from THS 2.2 for up to eight months does not increase cardiovascular disease, inflammation, and emphysema. Results are reproducible across two studies conducted by PMI.
- Switching from cigarette smoke exposure after two months to fresh air exposure or THS 2.2 aerosol exposure resulted in a partial (lung function, plaque area, lung morphometry) or even complete (pulmonary inflammation) recovery to sham-exposed levels.

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

Phillips B, Veljkovic E , Boué S , Schlage WK, Vuillaume G, Martin F, Titz B , Leroy P, Buettner A, Elamin A, Oviedo A, Cabanski M, Guedj E, Schneider T, Talikka M, Ivanov NV , Vanscheeuwijck P, Peitsch MC, Hoeng J.

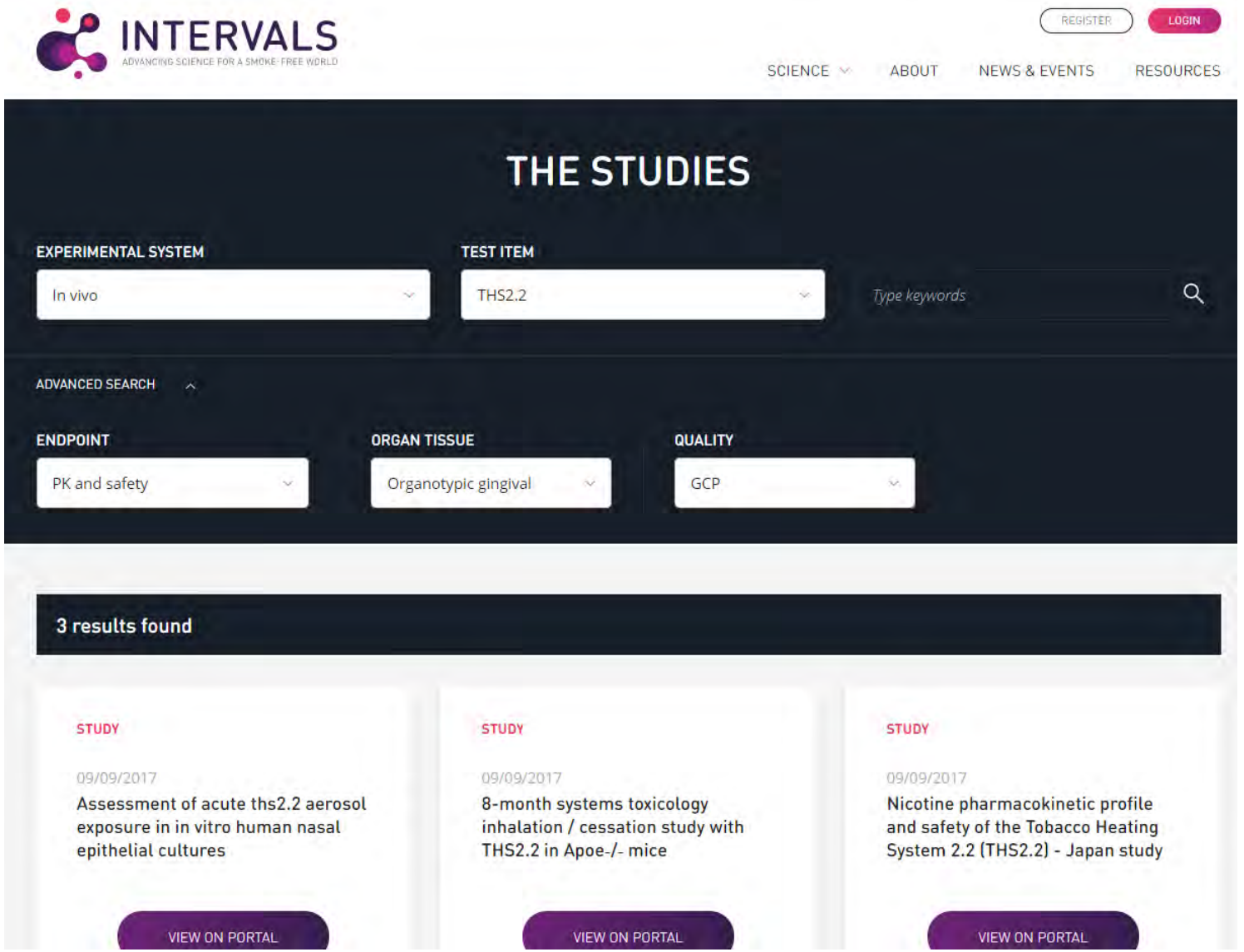
Toxicological Sciences, 149: 411-432 (2016)

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

INTERVALS - enabling science to support designing a smoke-free future

- Reproducible assessment of alternative products
- Enable evidence-based decisions

Overview of the Platform



- Faceted search enables quick retrieval of resource of interest
- Detailed protocols
- Study data sets
- Community features (news/commenting/events)

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Detailed Study Results and Direct Link to Data

Micro-CT at month 7

The additional quantitative micro-CT investigation of the aortic arch plaque formation *in situ* at the 7-month time-point **confirmed the morphometric results from the plaque surface assessment**: for 3R4F-exposed mice, all 3 parameters (plaque volume, plaque area, and aortic occlusion) were significantly higher compared with sham-exposed mice, but the THS2.2, cessation, and switching groups were not different from sham (see Figure 2 and videos below). The aorta plaque surface area (the micro-CT parameter most closely resembling the morphometric plaque area) was 78% higher for the 3R4F group versus sham, while manual quantification of plaque area in the isolated aortas showed a 39% higher value following 3R4F CS exposure.

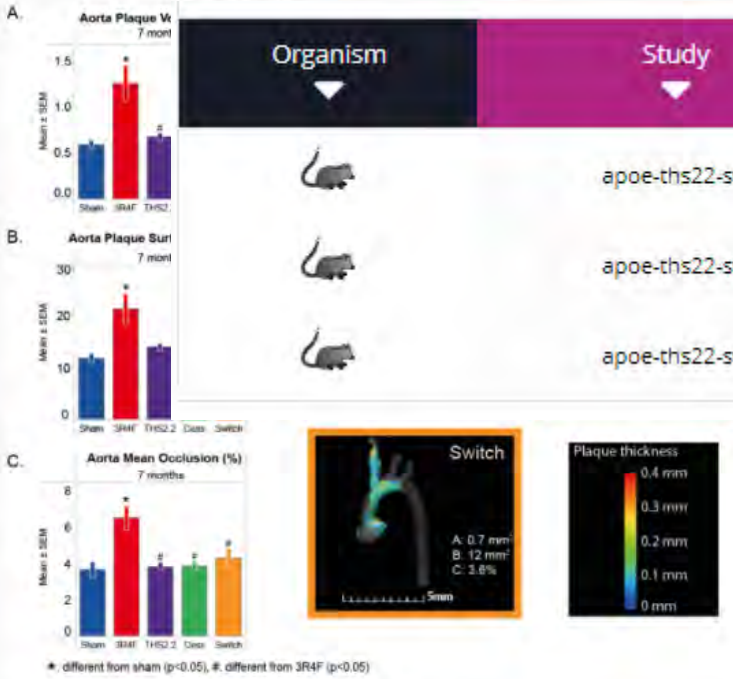

Figure 2 - Micro computed tomography (micro-CT)-based aortic arch (*in situ*) plaque measurements.
A, Plaque volume. B, Plaque surface area. C, Aortic occlusion (mean ± SEM). D, Representative micro-CT images.

Method: Plaque size measurements - planimetry and microCT

Planimetry

After removal of the aortic arch, the aortic wall was opened longitudinally, stained with Oil Red O, and the intimal area covered by plaques normalized to the whole area was determined from digital images. The intimal area covered by plaques was determined by planimetry and the values were normalized to the whole aortic arch area.

Organism	Study	Tissue	Endpoint (Species/tissue - Test item)	Data Download: PROCESSED
	apoe-ths22-sw	Aorta	Plaque size (Mm - THS2.2)	588.9 kB
	apoe-ths22-sw	Aorta	Aorta transcriptomics (Mm - THS2.2)	26.6 MB
	apoe-ths22-sw	Aorta	Aorta lipidomics (Mm - THS2.2)	switch data type to access data

at each point along the aorta. At the bottom of this frame, the slice distance and plaque cross-sectional area are reported, as well as total measurements (average occlusion, total plaque volume, total plaque surface area) for each of the regions (sinus, aortic arch, thoracic aorta, brachiocephalic trunk).

- **Linear Distance Measurements (top-right)** – for each slice along the curved centerline, the average aorta radius, maximum plaque thickness, and average plaque thickness are plotted. As the animation proceeds, a black time-bar indicates the current slice distance along the graph.
- **Percent Measurements (middle-right)** – For each slice along the curved centerline, the percent coverage (percent of the vessel wall that has plaque attached) and percent occlusion (percent of the vessel cross-section that is occluded with plaque) are plotted. As the animation proceeds, a black time-bar indicates the current slice distance along the graph.
- **Two planar slices (bottom-right)** – the grayscale slices cut through the aorta in an orientation centered around and perpendicular to the centerline. The right side is displayed with segmented aortic plaque overlaid in red, and segmented brachiocephalic trunk plaque overlaid in blue.

All metrics and 3D movies were created for the aortas using SCIRun (Scientific Computing and Imaging Institute, University of Utah). All samples were scanned and analyzed blind to treatment assignment.

Thank you for your attention!

Collaborators

The Singapore Team