Molecular biomarkers elucidate the role of inflammation and oxidative stress in smoking-related diseases

17 May 2018

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Faculty Disclosure

	No, nothing to disclose
X	Yes, please specify:

Company Name	*Honoraria/ Expenses*	*Consulting/ Advisory Board*	*Funded Research*	*Royalties/ Patent*	*Stock Options*	*Ownership/ Equity Position*	*Employee*	*Other (please specify)*
Philip Morris International							X	

PMI's goal for harm reduction

Offering adult smokers satisfying products that reduce risk

- 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 (RRP) so that consumer acceptance can be best fulfilled

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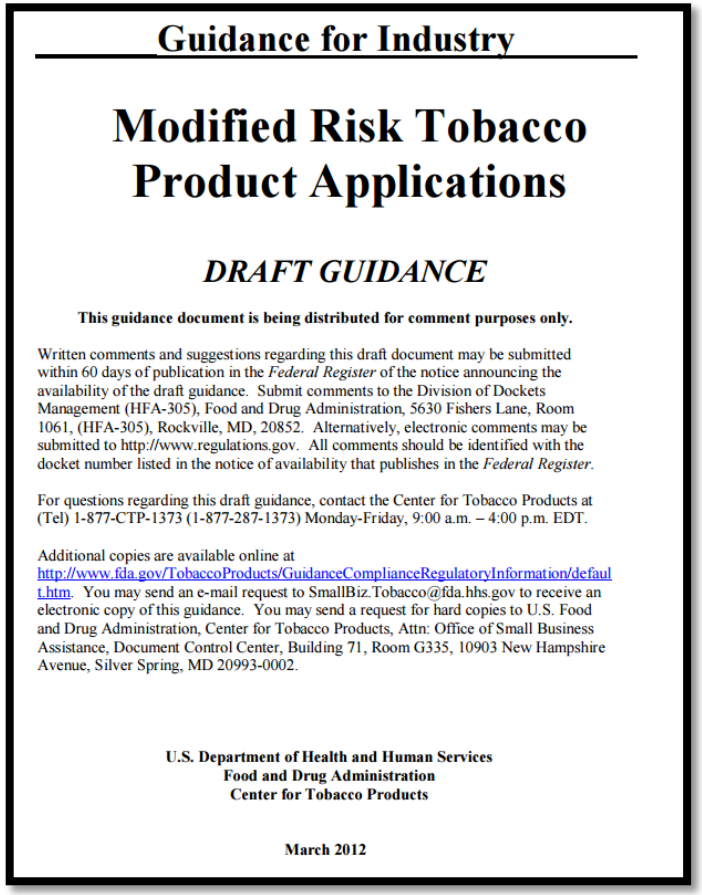
*The Tobacco Atlas 3rd Edition. American Cancer Society, 2009
Figure adapted from Clive Bates presentation to E-Cigarette Summit (19 Nov 2013)

What is an RRP?

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 RRPs do not burn tobacco, they produce far lower quantities of harmful and potentially harmful compounds than found in cigarette smoke.**



Guidance for Industry

Modified Risk Tobacco Product Applications

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Written comments and suggestions regarding this draft document may be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. Alternatively, electronic comments may be submitted to http://www.regulations.gov. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft guidance, contact the Center for Tobacco Products at (Tel) 1-877-CTP-1373 (1-877-287-1373) Monday-Friday, 9:00 a.m. – 4:00 p.m. EDT.

Additional copies are available online at http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/default.htm. You may send an e-mail request to SmallBiz.Tobacco@fda.hhs.gov to receive an electronic copy of this guidance. You may send a request for hard copies to U.S. Food and Drug Administration, Center for Tobacco Products, Attn: Office of Small Business Assistance, Document Control Center, Building 71, Room G335, 10903 New Hampshire Avenue, Silver Spring, MD 20993-0002.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Tobacco Products

March 2012

https://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297751.pdf

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Two case studies

- Comparative assessment of lung inflammation, pulmonary function, and emphysema caused by the aerosol from potential Reduced Risk Products and cigarette smoke in mouse models of COPD.

- Summary of ambulatory exposure clinical ZRHM-REXA-07-JP study results

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Case study: Comparative assessment of lung inflammation, pulmonary function, and emphysema caused by the aerosol from potential Reduced Risk Products and cigarette smoke in mouse models of COPD.

This research was funded by Philip Morris International

Aim and scope of the presentation

- Assessment of the effects of conventional cigarette smoke and RRP, using PMI's Heat-not-Burn technology, in an animal model of chronic obstructive pulmonary disease (COPD):
  — ApoE$^{-/-}$ mouse (C57Bl6 background), typically used as model for cardiovascular disease

- The animal model is responsive to cigarette mainstream smoke and develop different pathologies, among which aspects of COPD, such as *lung inflammation*, changed *pulmonary function*, *emphysema**

*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**

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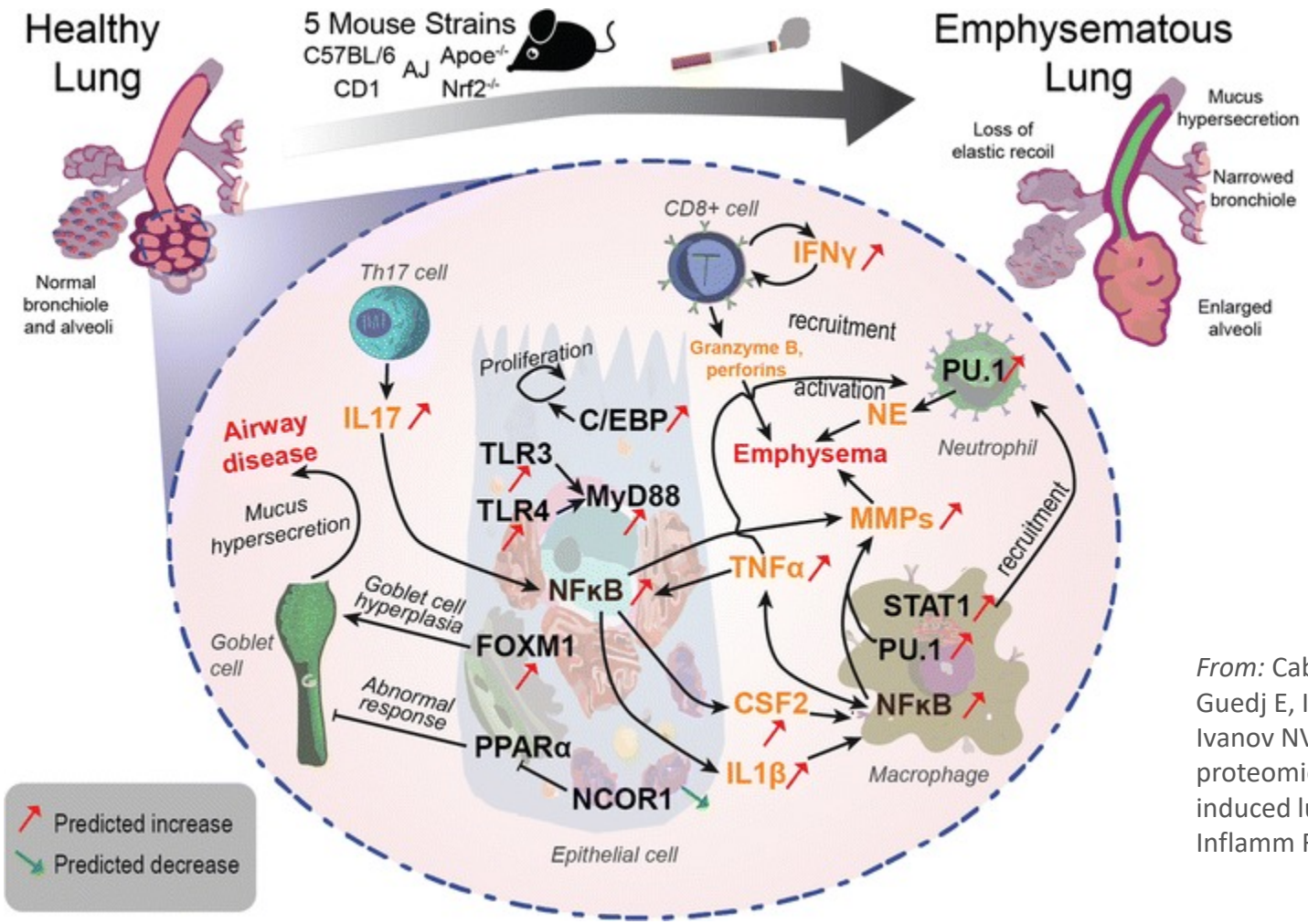
Common disease mechanisms in different mouse models, relevance to human situation



- 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*)

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*

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Methods – ~~Conventional~~ cigarette smoke and aerosol from an RRP

Assessment of smoke/aerosol:
Health Canada Intense smoke protocol

Smoke from University of Kentucky Standard Reference Cigarette 3R4F

Potential RRP:
Aerosol from HeatSticks and Tobacco Heating System (THS) 2.2

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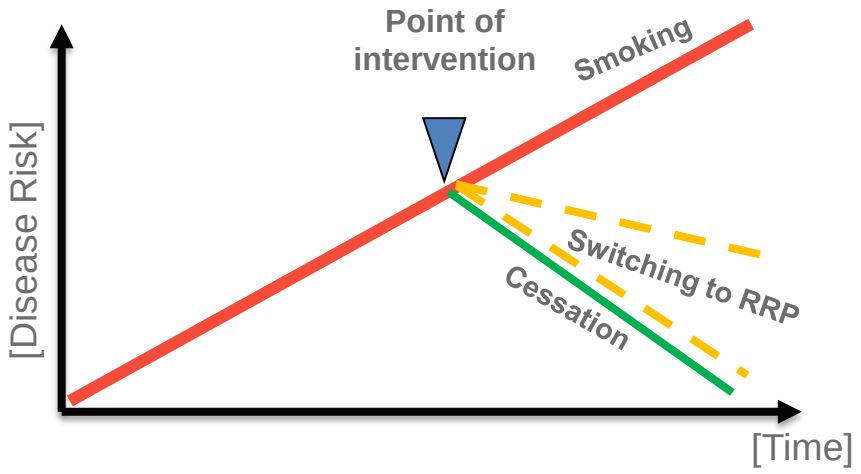
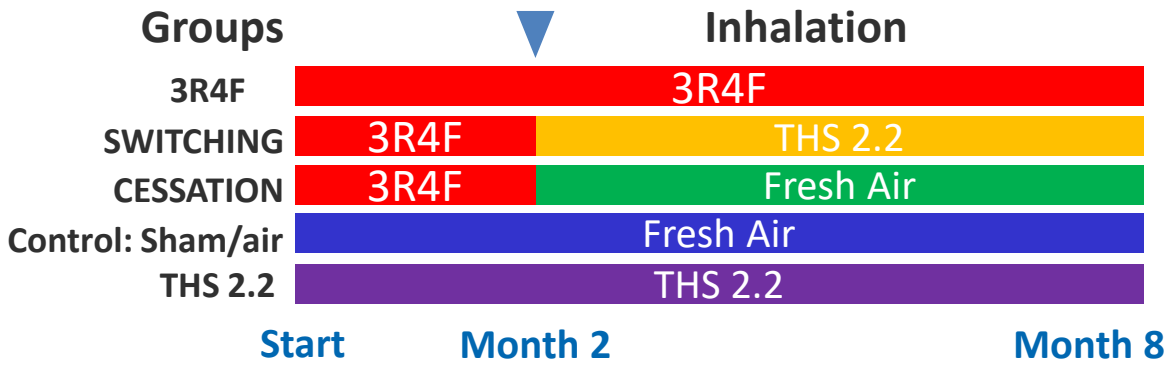
ApoE$^{-/-}$ mouse switching study
Study design

- Comparative assessment of effects of THS 2.2 and 3R4F
- Switching design upon initiation of disease:
  - To assess reversibility (switch to fresh air, i.e., cessation)
  - To quantify how similar switching to THS 2.2 is to cessation





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

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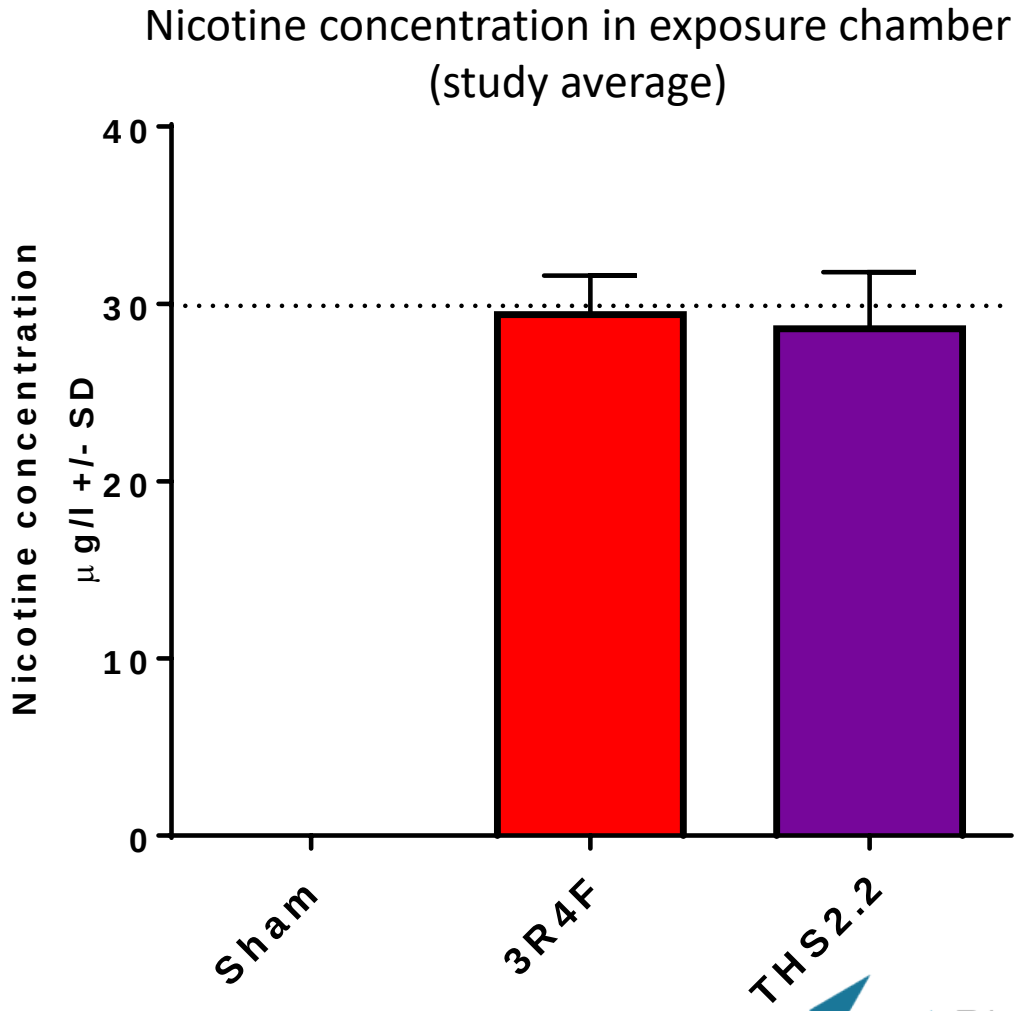
ApoE$^{-/-}$ mouse switching study
Methods - exposure regime

1 hour	30 min	1 hour	1 hour	1 hour

Fresh air

THS 2.2 or 3R4F

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



*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

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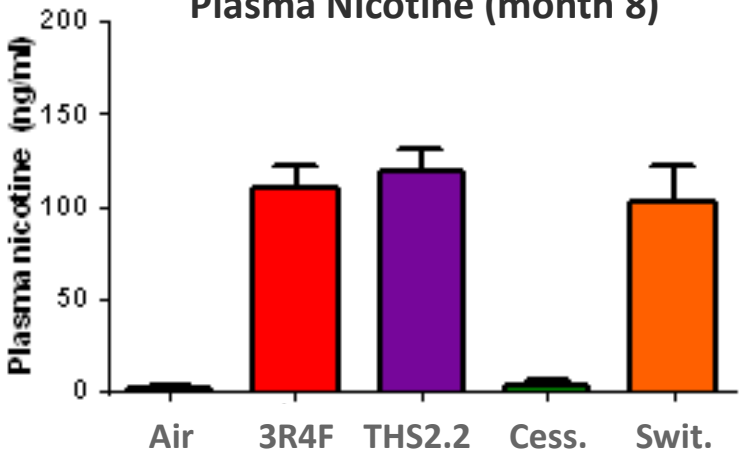
ApoE$^{-/-}$ mouse switching study
Aerosol uptake (biomarkers of exposure)

Plasma Nicotine (month 8)



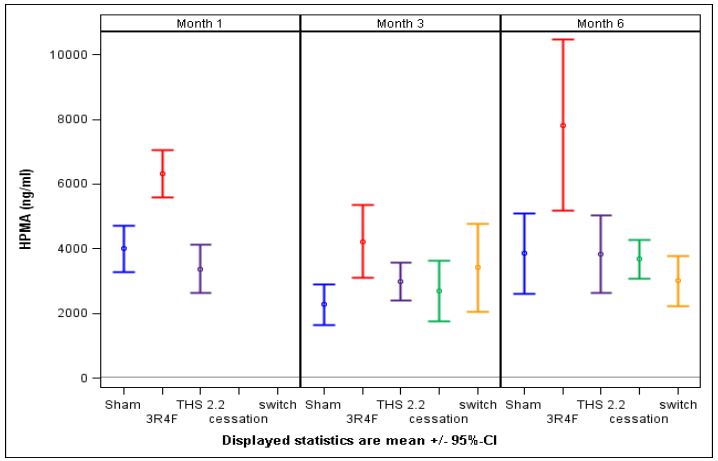
Means ± SEM

Groups

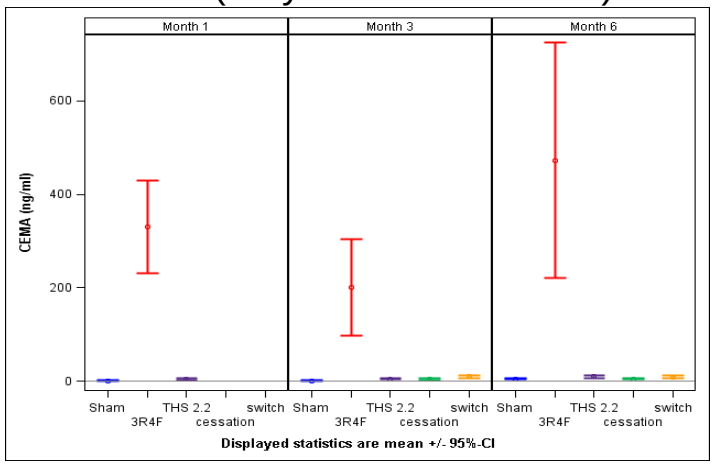
- 3R4F
- Cessation
- Switching
- THS 2.2
- Sham / air

Urinary Metabolites (Months 3, 6, 8)

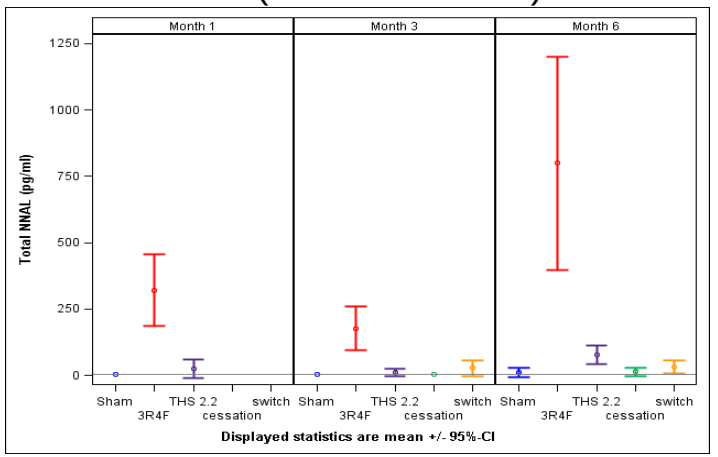
HPMA (Acrolein metabolite)



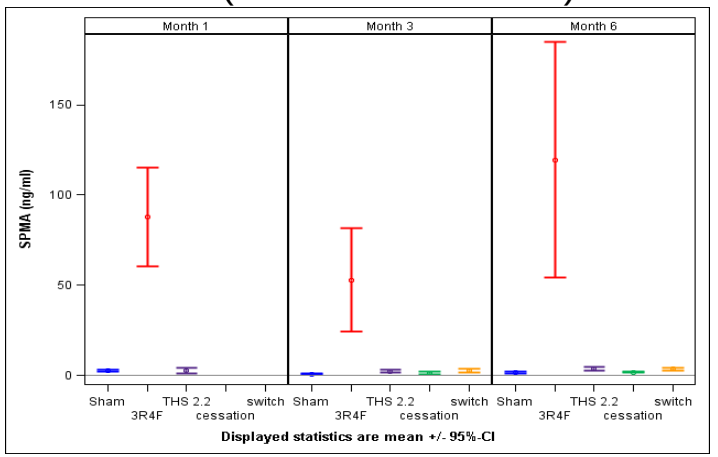
CEMA (Acrylonitrile metabolite)



NNAL (NNK metabolite)



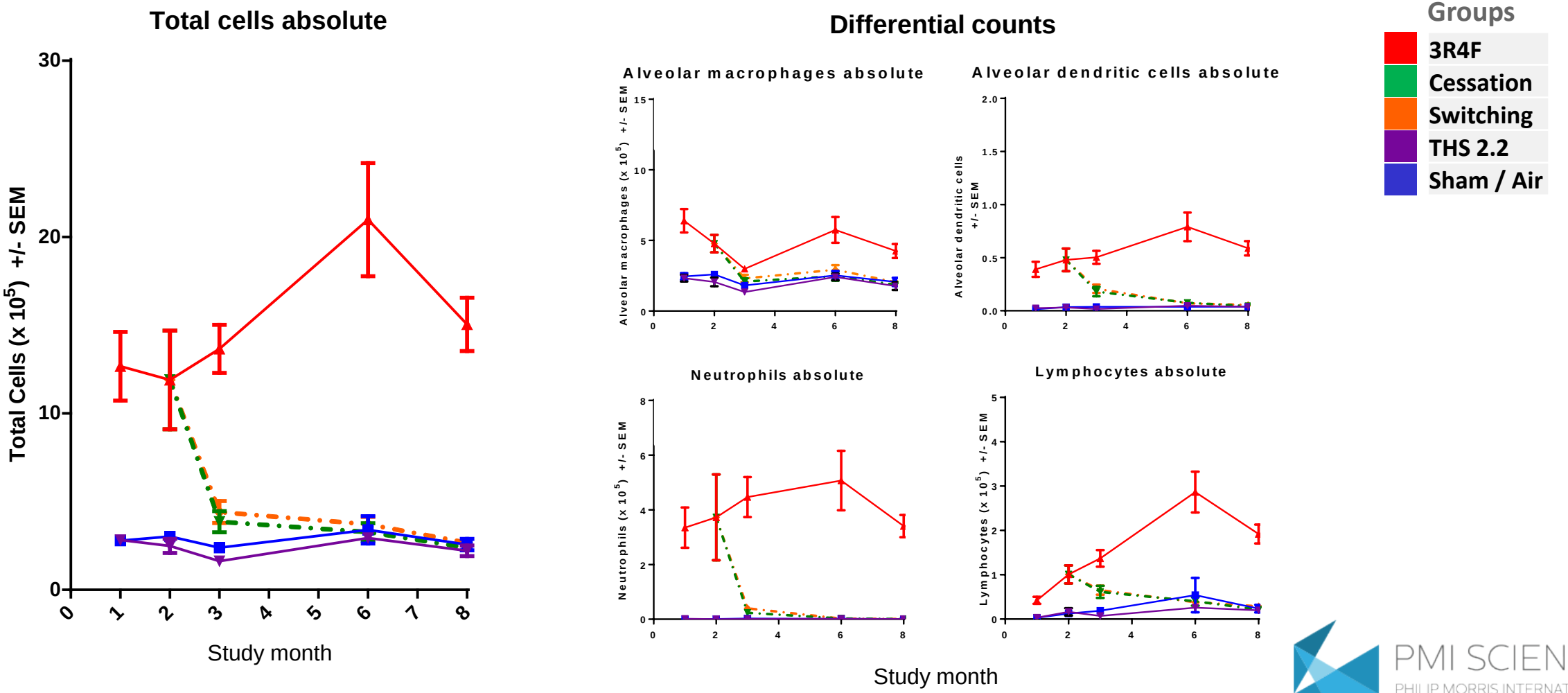
SPMA (Benzene metabolite)



Means ± SEM

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

Free lung cells in bronchoalveolar lavage fluid (BALF)



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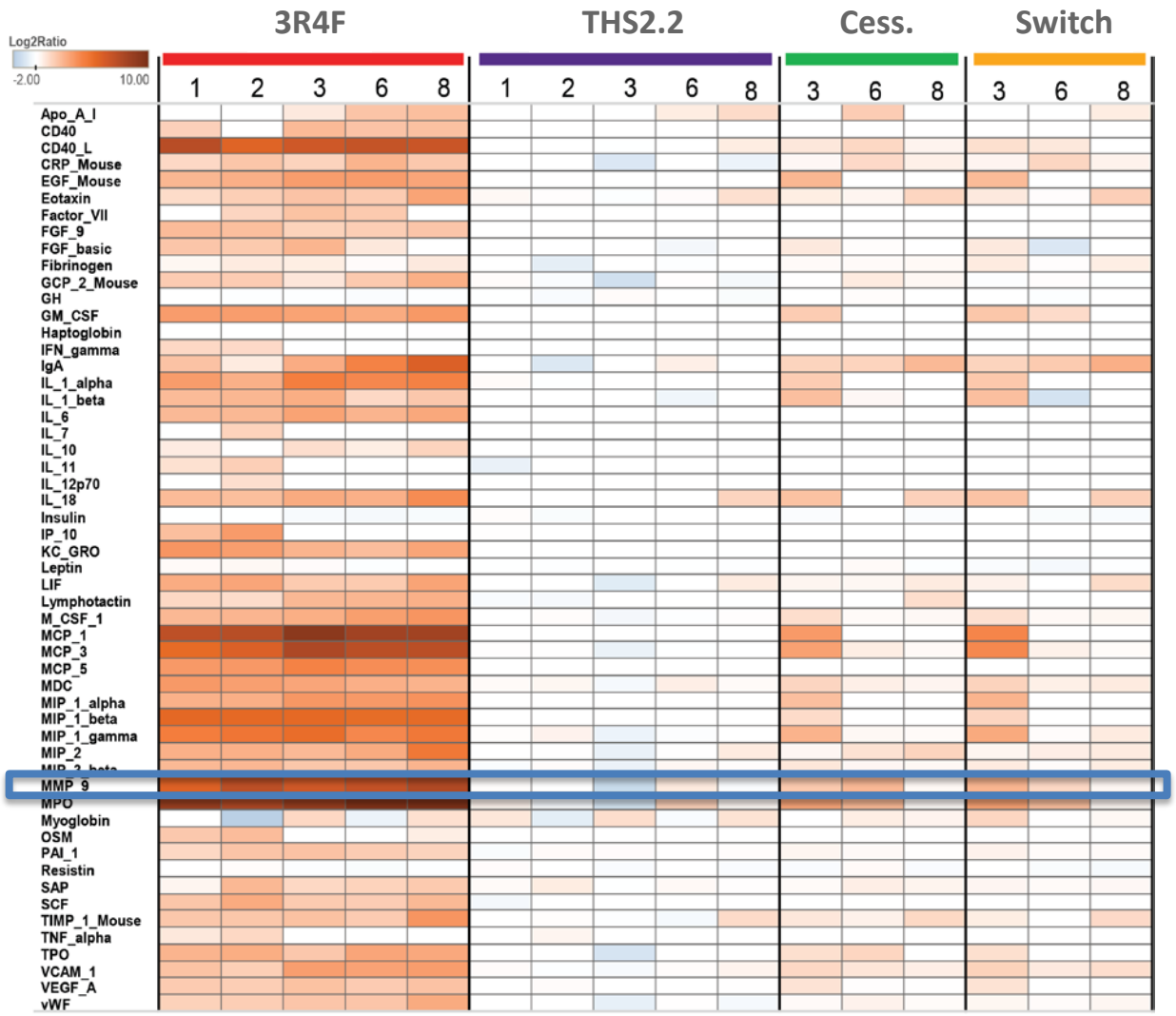
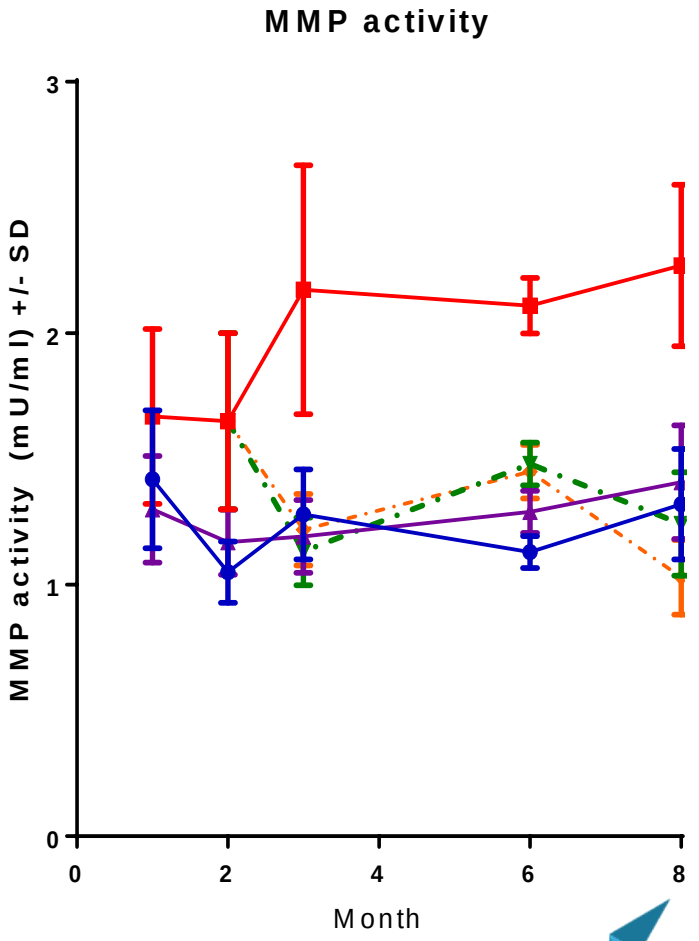
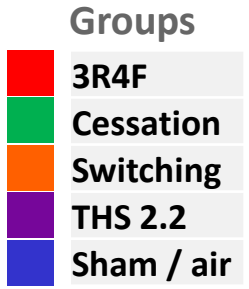
ApoE$^{-/-}$ mouse switching study
Result summary: Disease mechanisms - lung inflammation

Multiple analyte profiling in BALF







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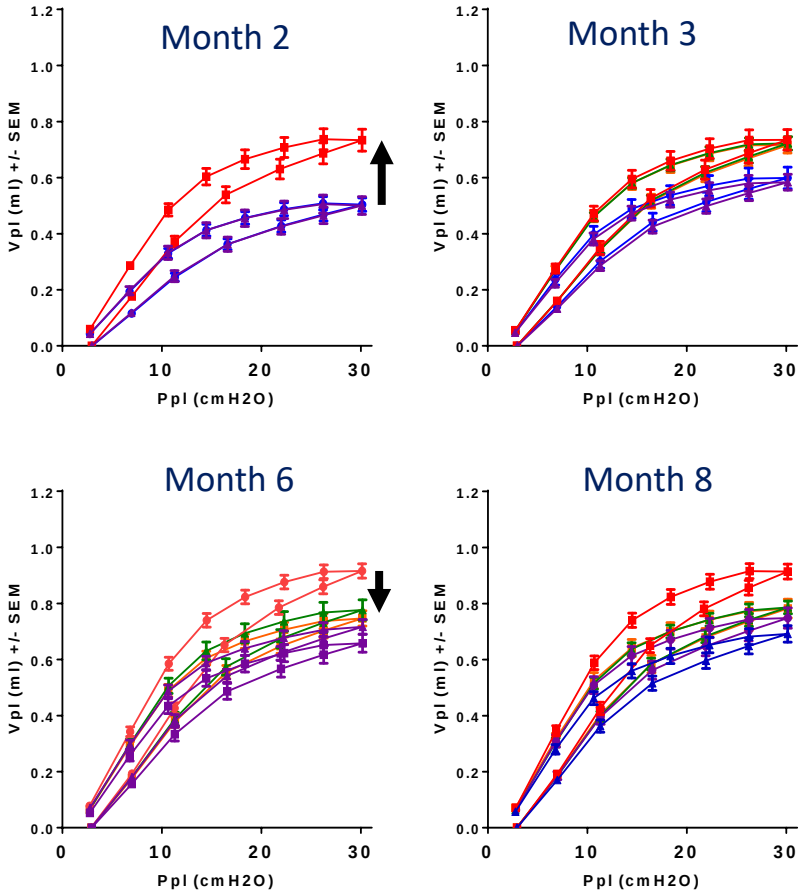
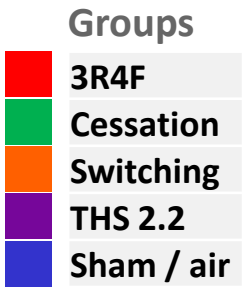
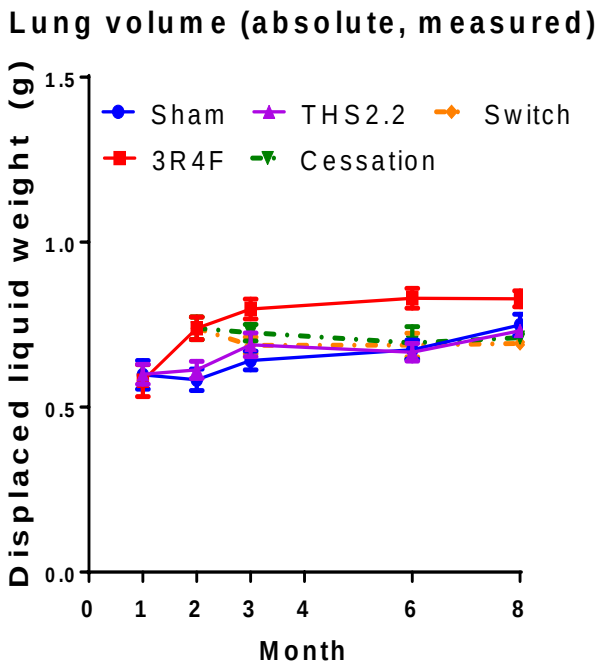
ApoE$^{-/-}$ mouse switching study
Result summary: Disease endpoints - lung function and lung volume

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



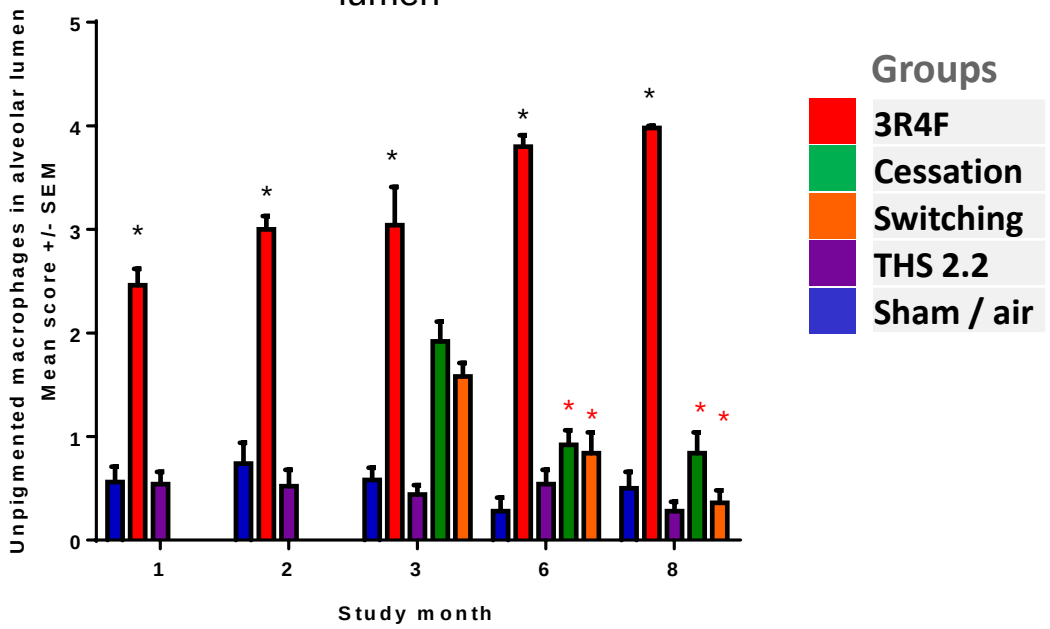




Means ± SEM

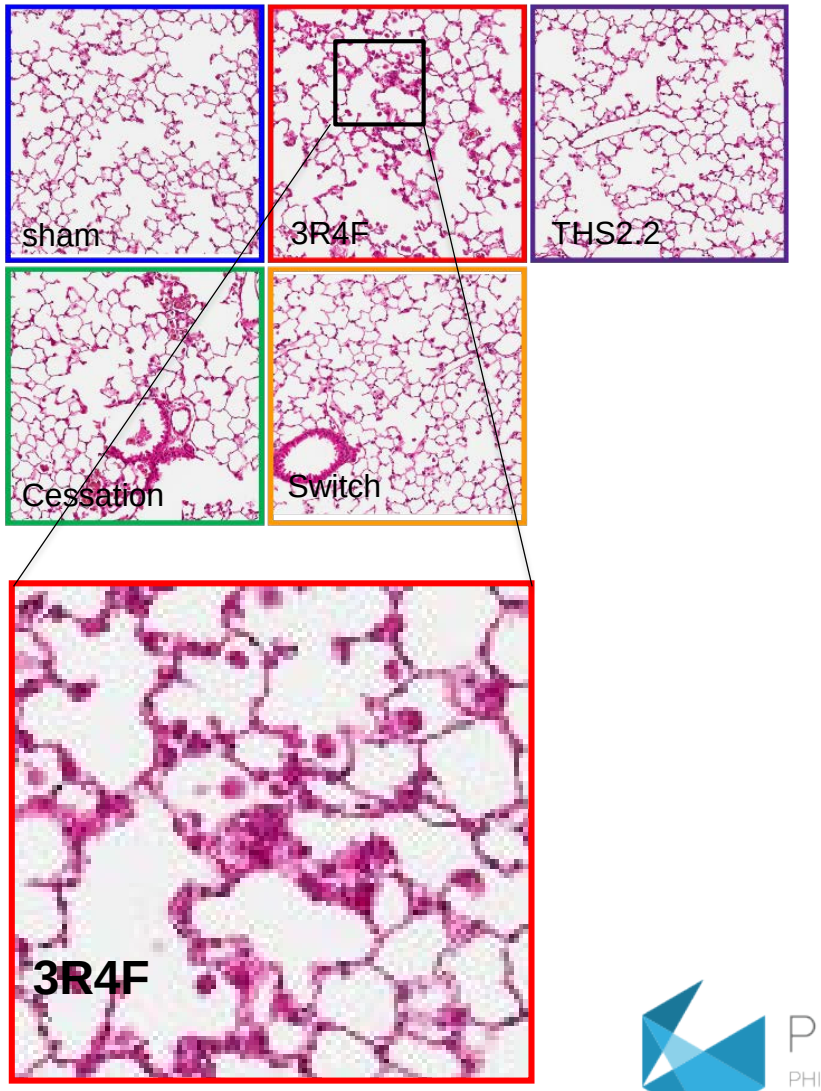
ApoE$^{-/-}$ mouse switching study
Result summary: Histopathology of the lung – pulmonary inflammation

Results, lung inflammation
unpigmented macrophages in the alveolar lumen



*: Statistically significant compared to sham
*: Statistically significant compared to 3R4F at Month 2

- Decrease in mean scores after switching to fresh air or THS 2.2 (statistically significant from Month 6)
- No statistically significant difference between Cessation group and THS 2.2-Switch group at Month 3



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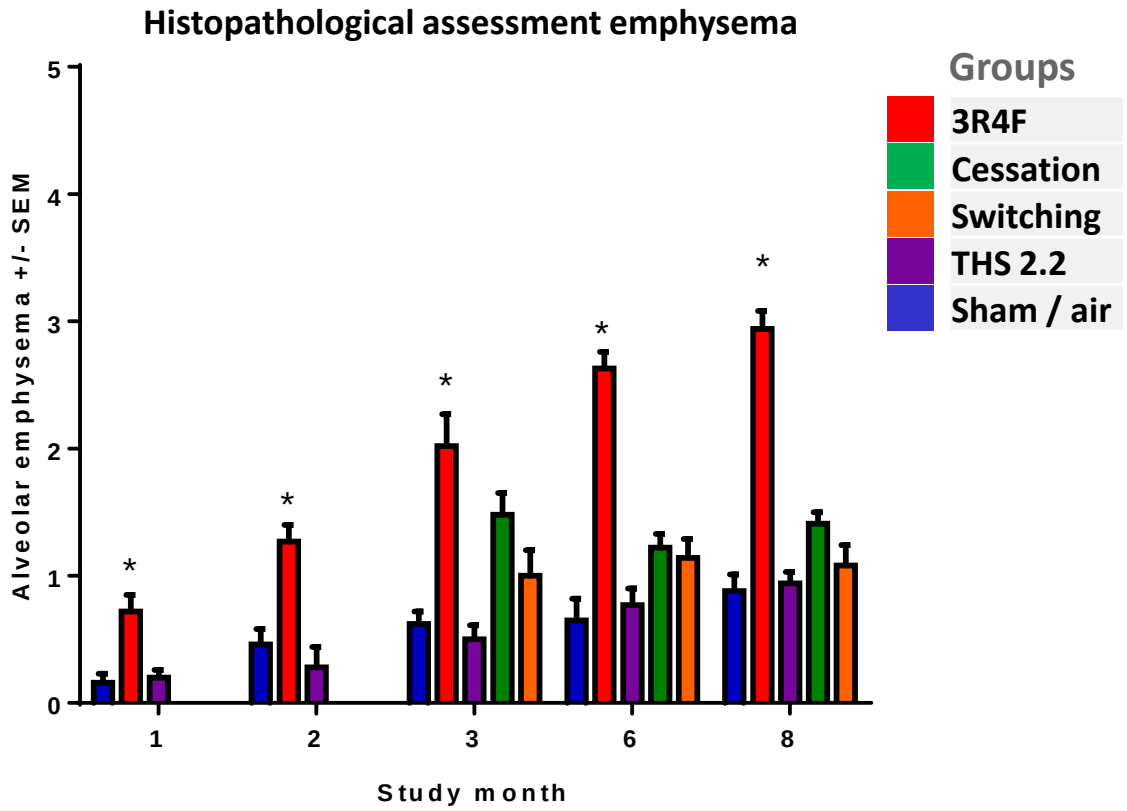
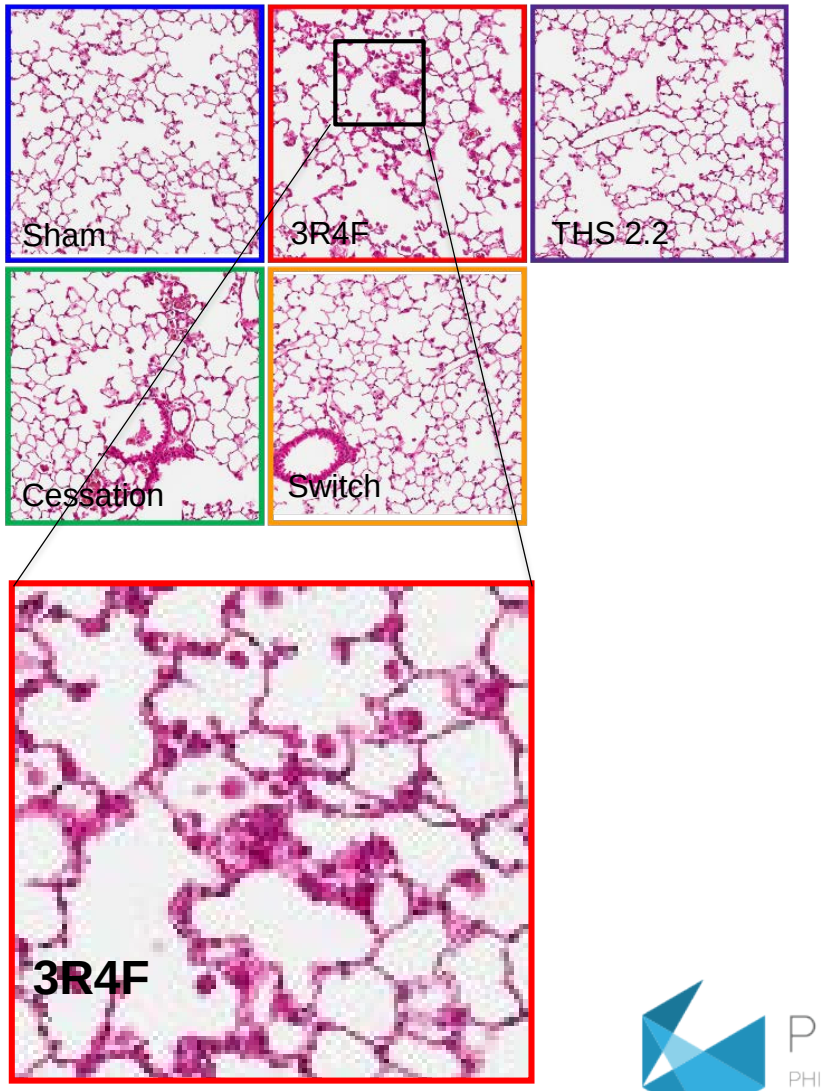
ApoE$^{-/-}$ mouse switching as an animal model of disease
Result summary: Tissue changes – histopathology



*: Statistically significant compared to sham



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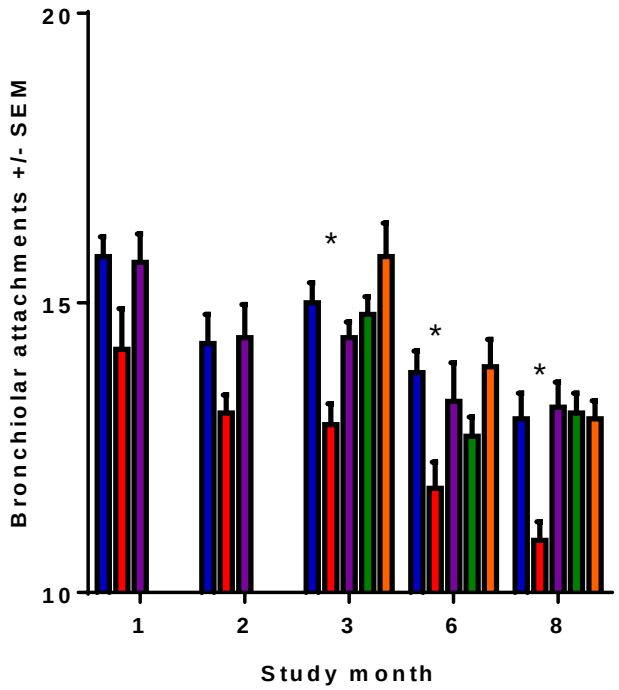
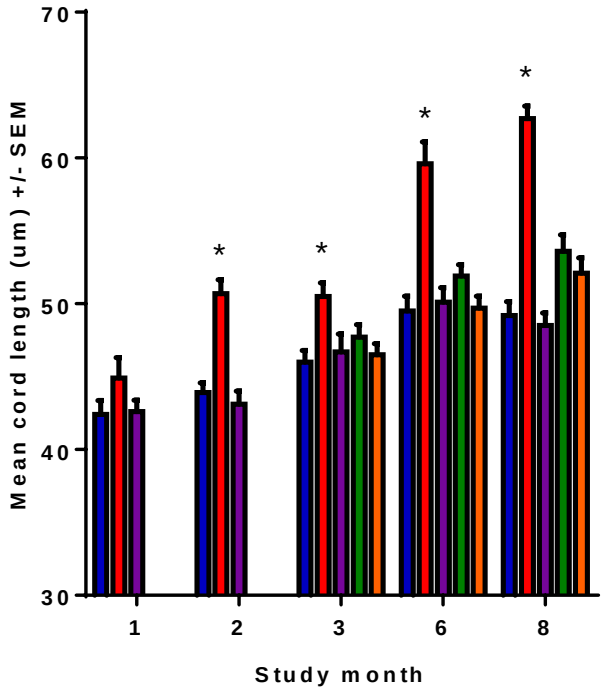
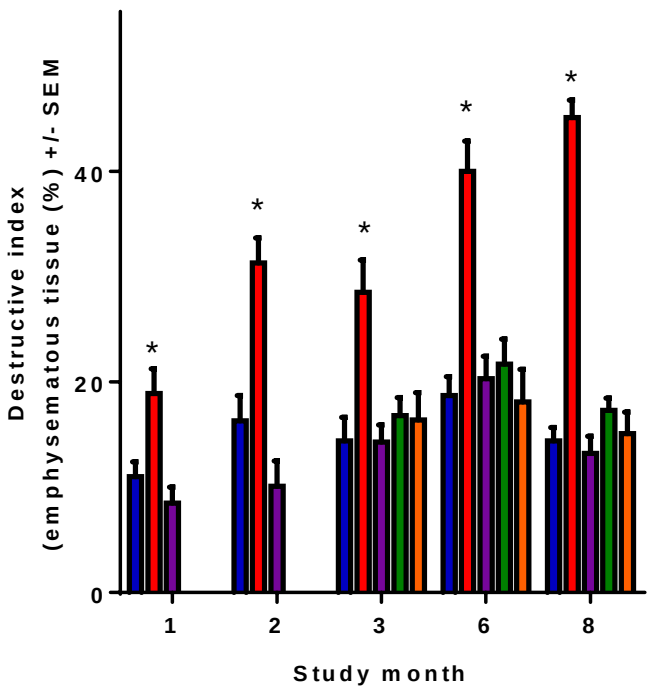
ApoE$^{-/-}$ mouse switching study
Result summary: Lung tissue changes – morphometry



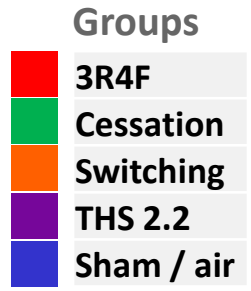
Bronchiolar attachments
- Fewer bronchiolar attachments in 3R4F-exposed group



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



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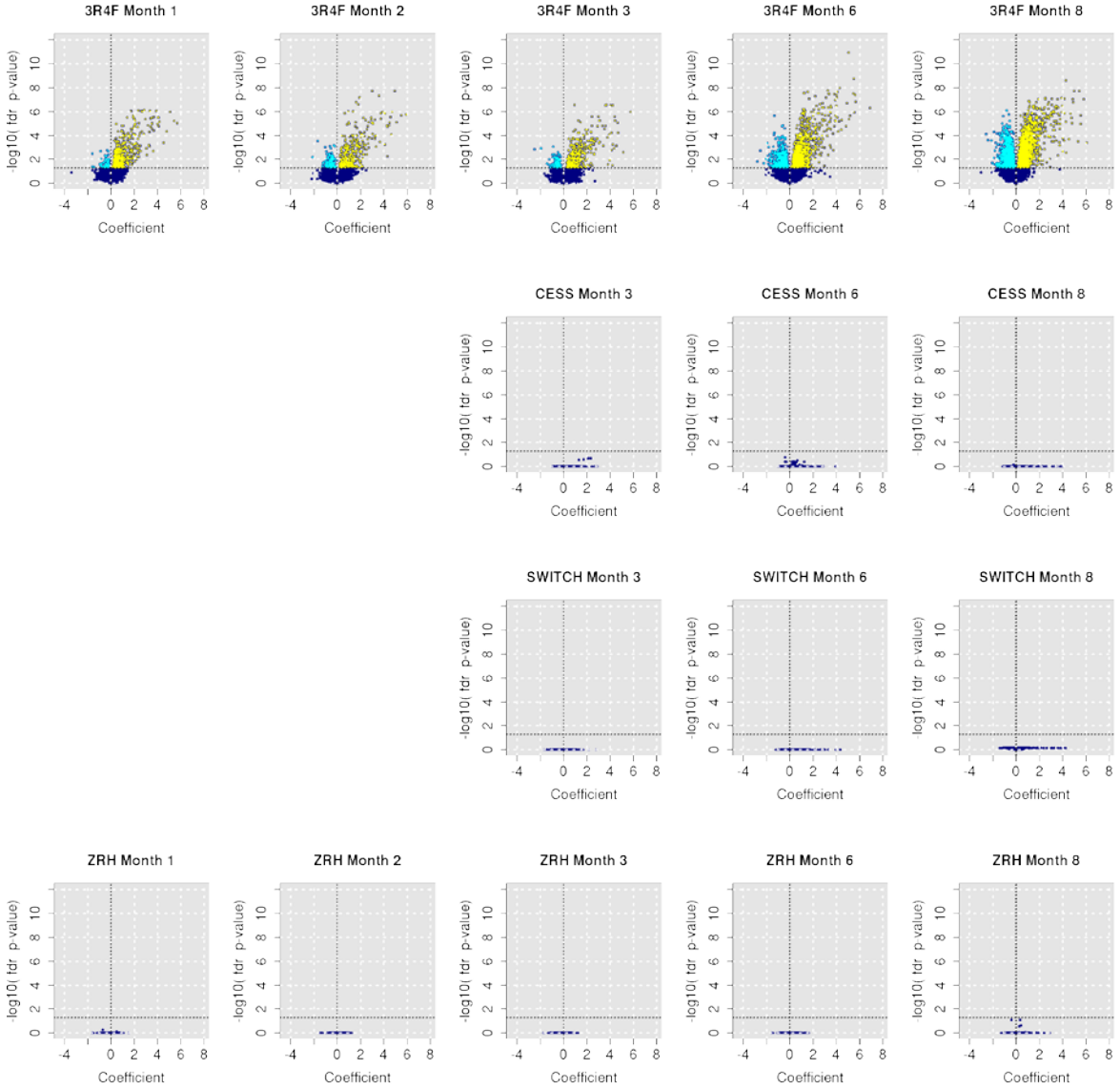
ApoE$^{-/-}$ mouse switching study
Result summary: Systems response profile - differential gene expression - lung

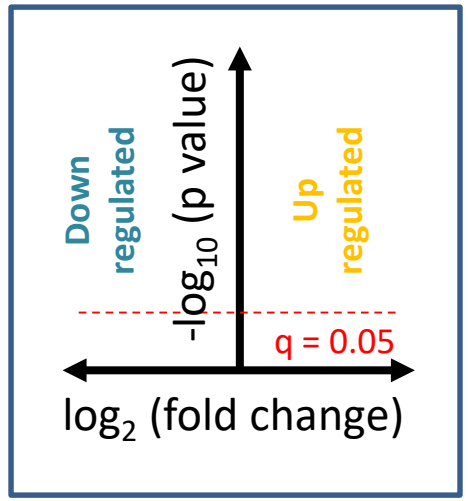
3R4F

Cessation

Switch

THS 2.2





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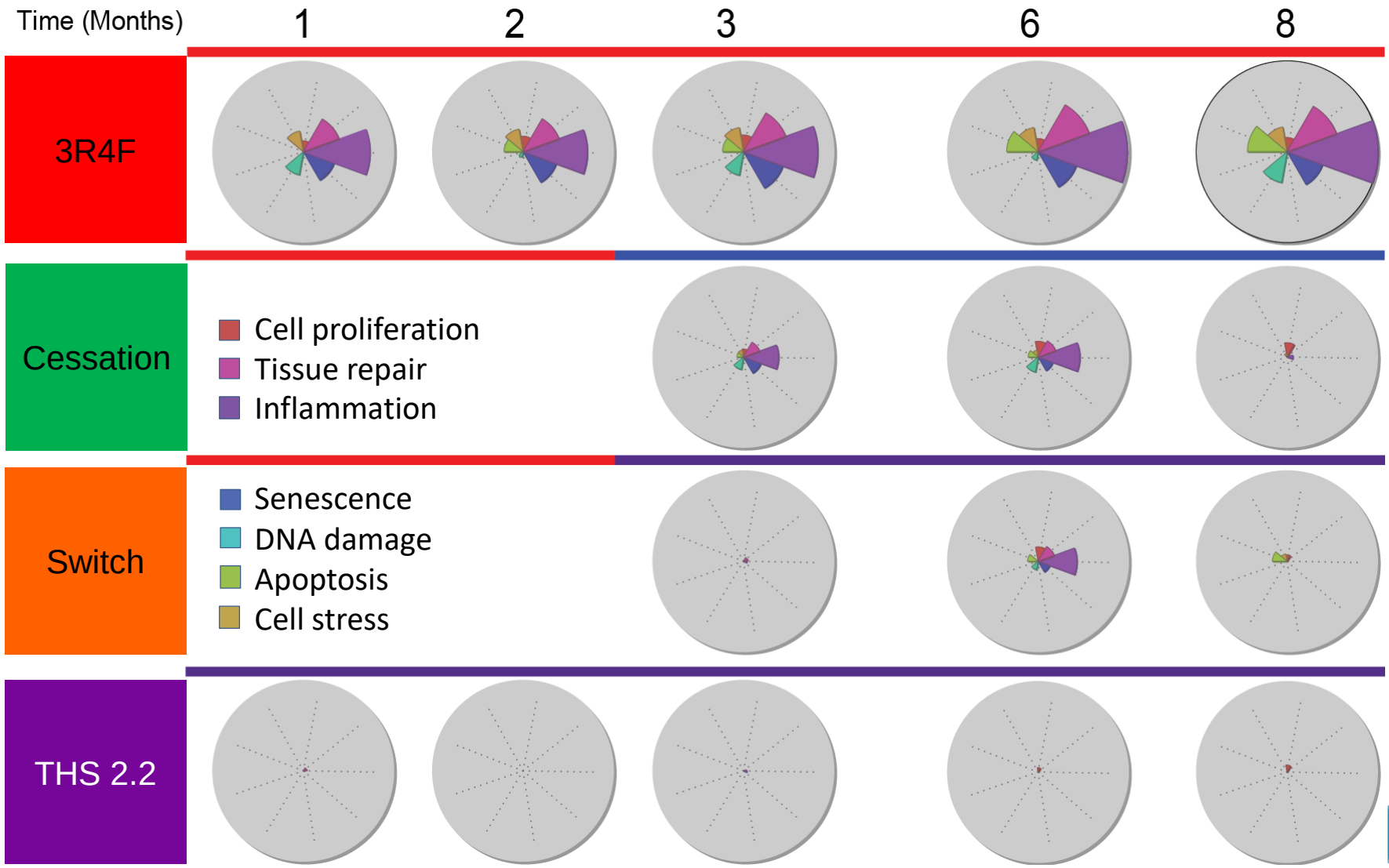
ApoE$^{-/-}$ mouse switching study
Result summary: Disease mechanisms - network perturbations - lung



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ApoE$^{-/-}$ mouse as 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 THS 2.2 for up to eight months does not increase inflammation and emphysema in comparison with the Sham group
- Switching from cigarette smoke exposure after two 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 THS 2.2 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**.

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Case study 2: Summary of ambulatory exposure clinical ZRHM-REXA-07-JP study results

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 (THS 2.2 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

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Study products and interventions

= THSm2.2

(Tobacco Heating System 2.2 menthol)

= mCC

(Menthol cigarettes)

= SA

(Smoking abstinence)

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Primary objective and endpoints

- To demonstrate the reduction of biomarkers of exposure (BoExp) to harmful and potentially harmful constituents (HPHC) in smokers switching from menthol cigarette (mCC) to THS 2.2 Menthol (THSm2.2) compared with smokers continuing to smoke mCC
- MHBMA, 3-HPMA, S-PMA, COHb after five days (confinement)
- Total NNAL after 90 days (ambulatory)

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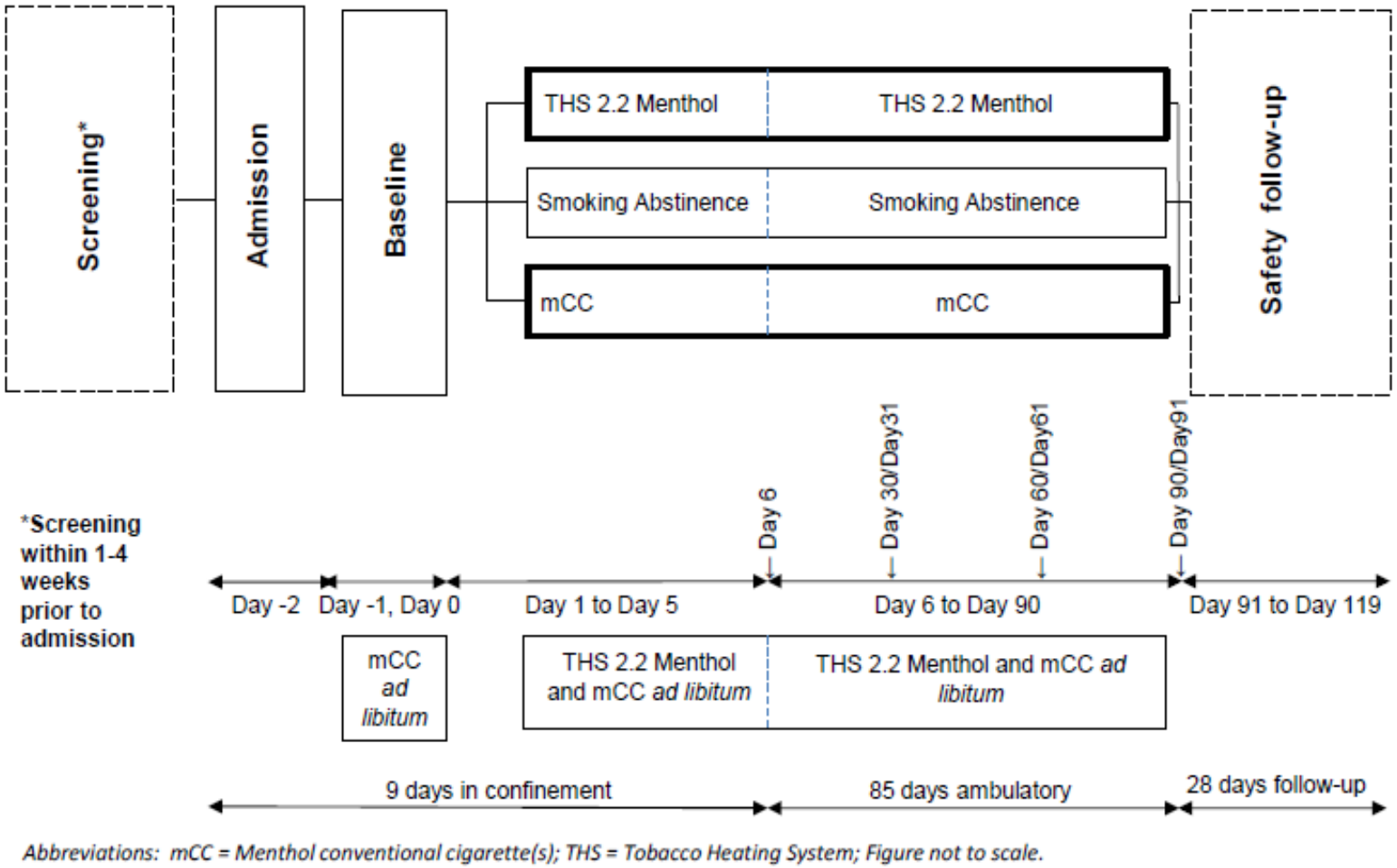
Additional objectives and endpoints

- 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

Study design



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

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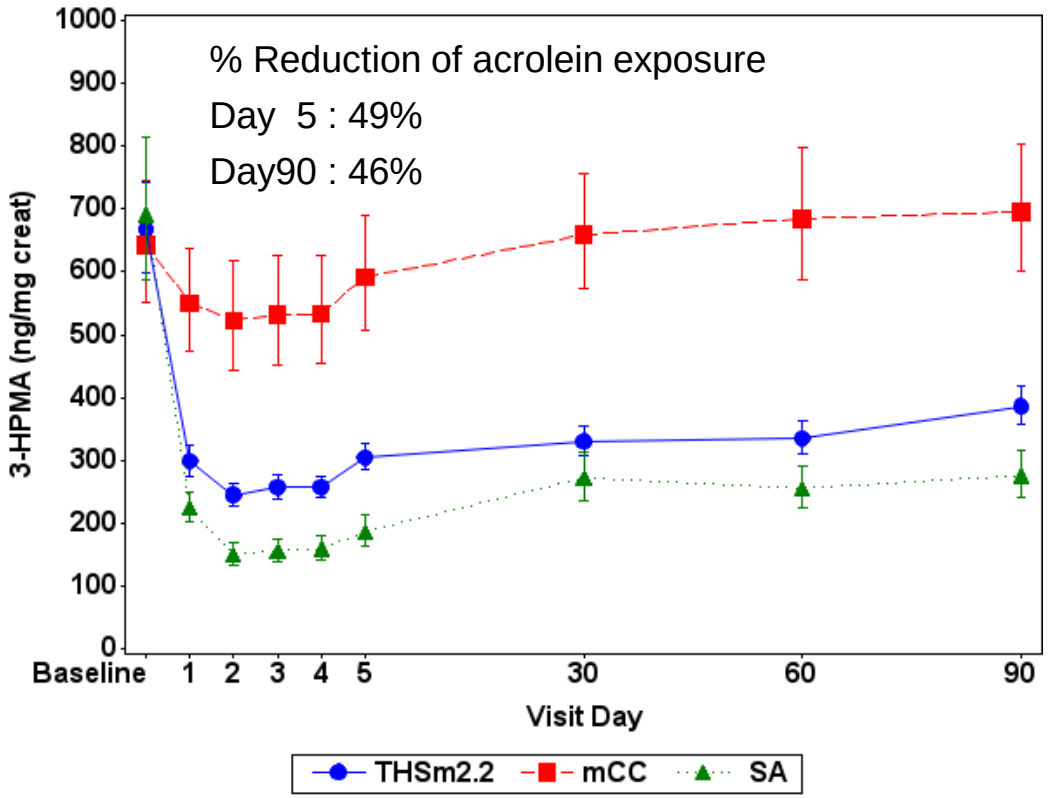
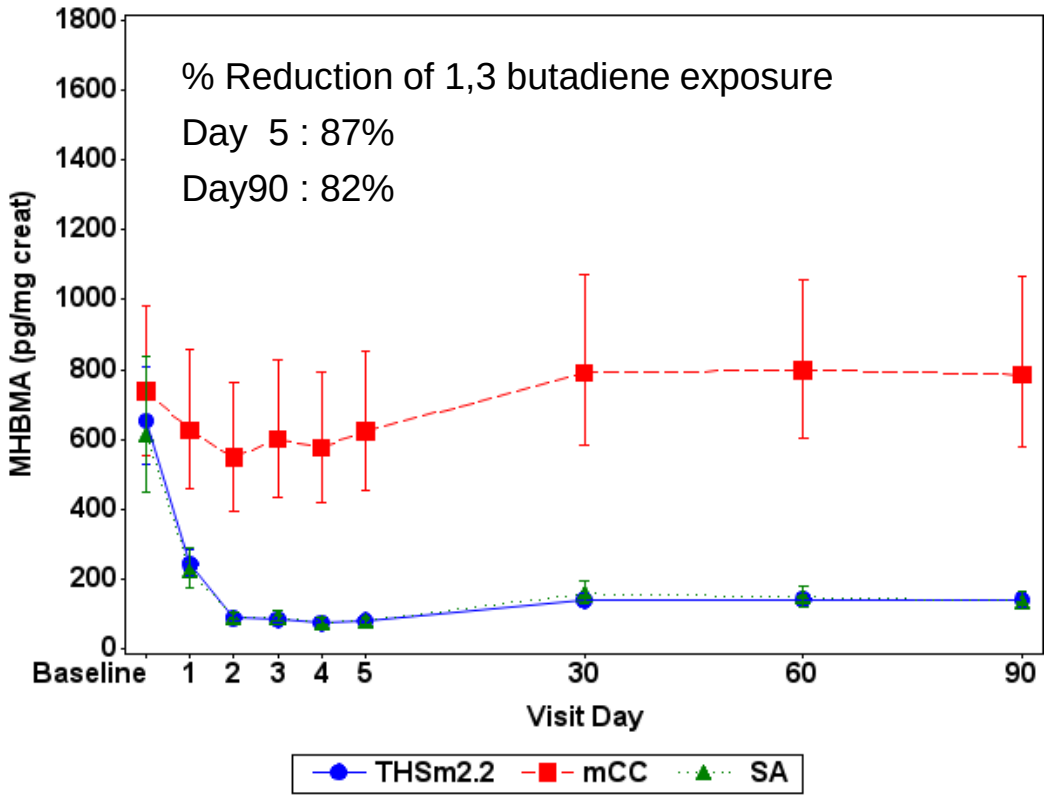
Japanese Population Characteristics

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

Biomarkers following 90 days of exposure (1/3)





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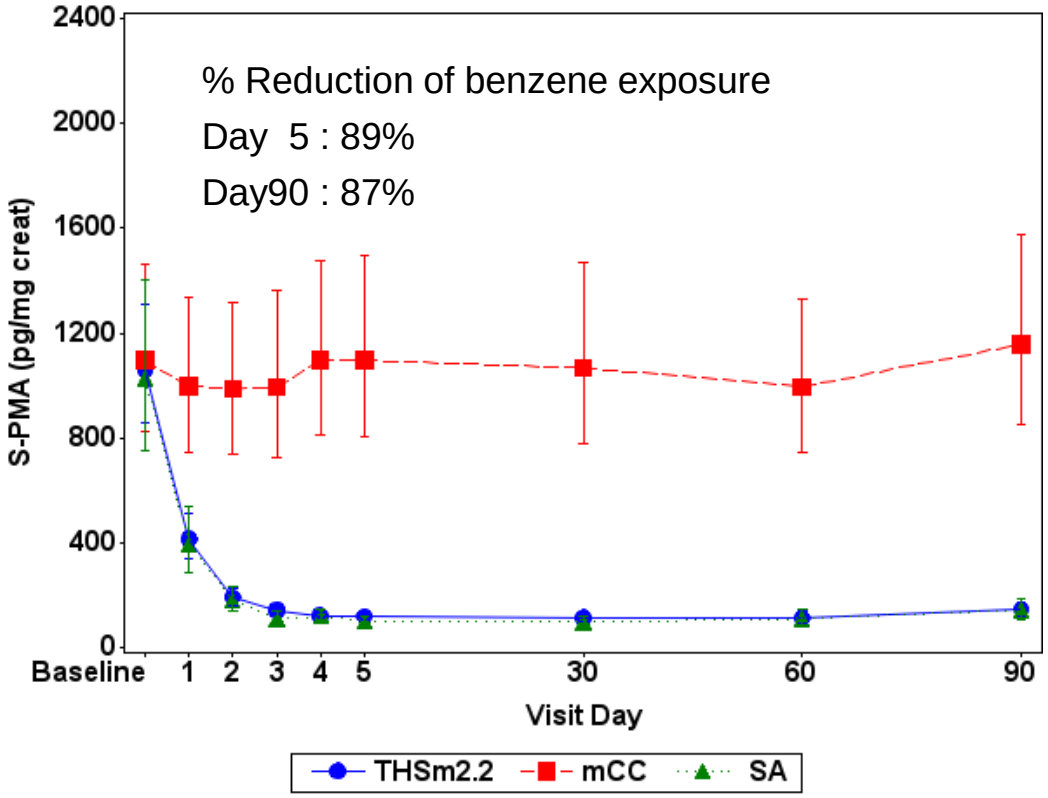
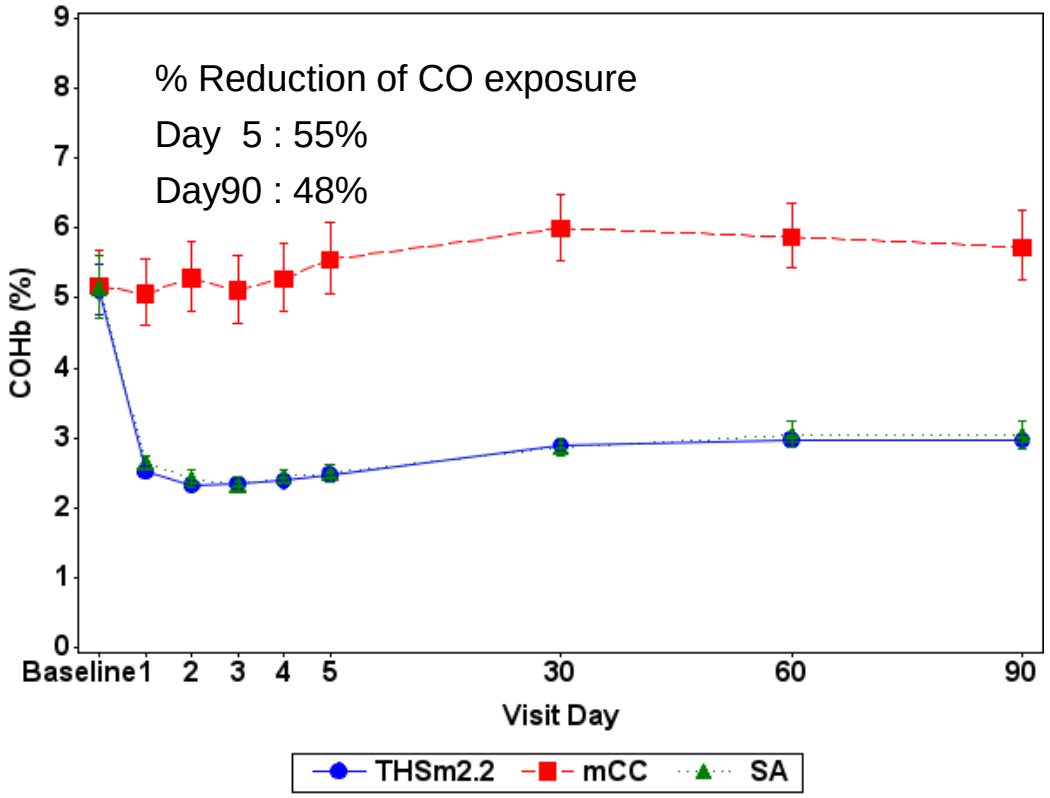
Biomarkers following 90 days of exposure (2/3)





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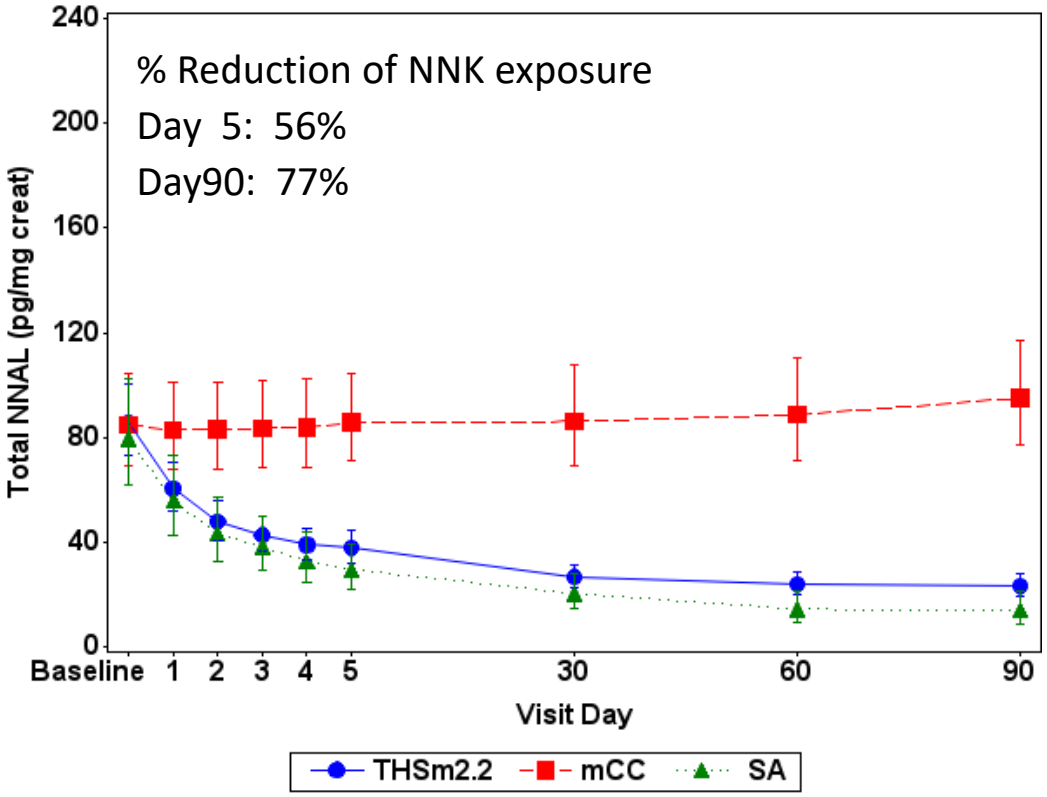
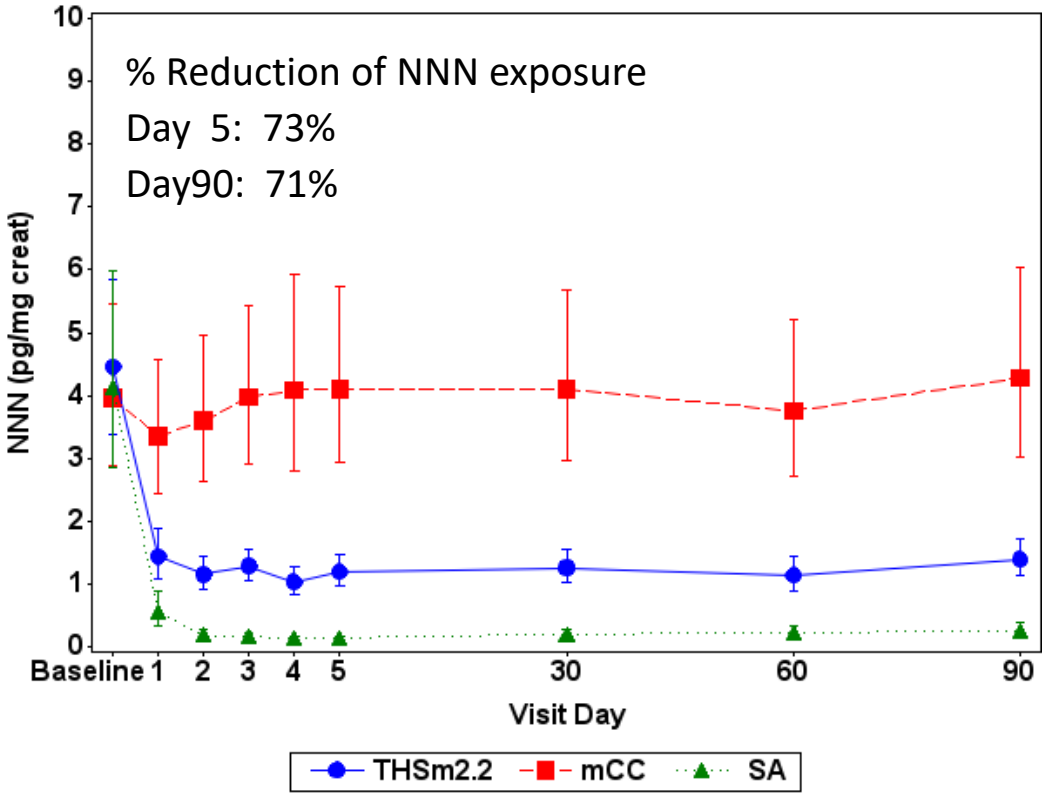
Biomarkers following 90 days of exposure (3/3)





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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)

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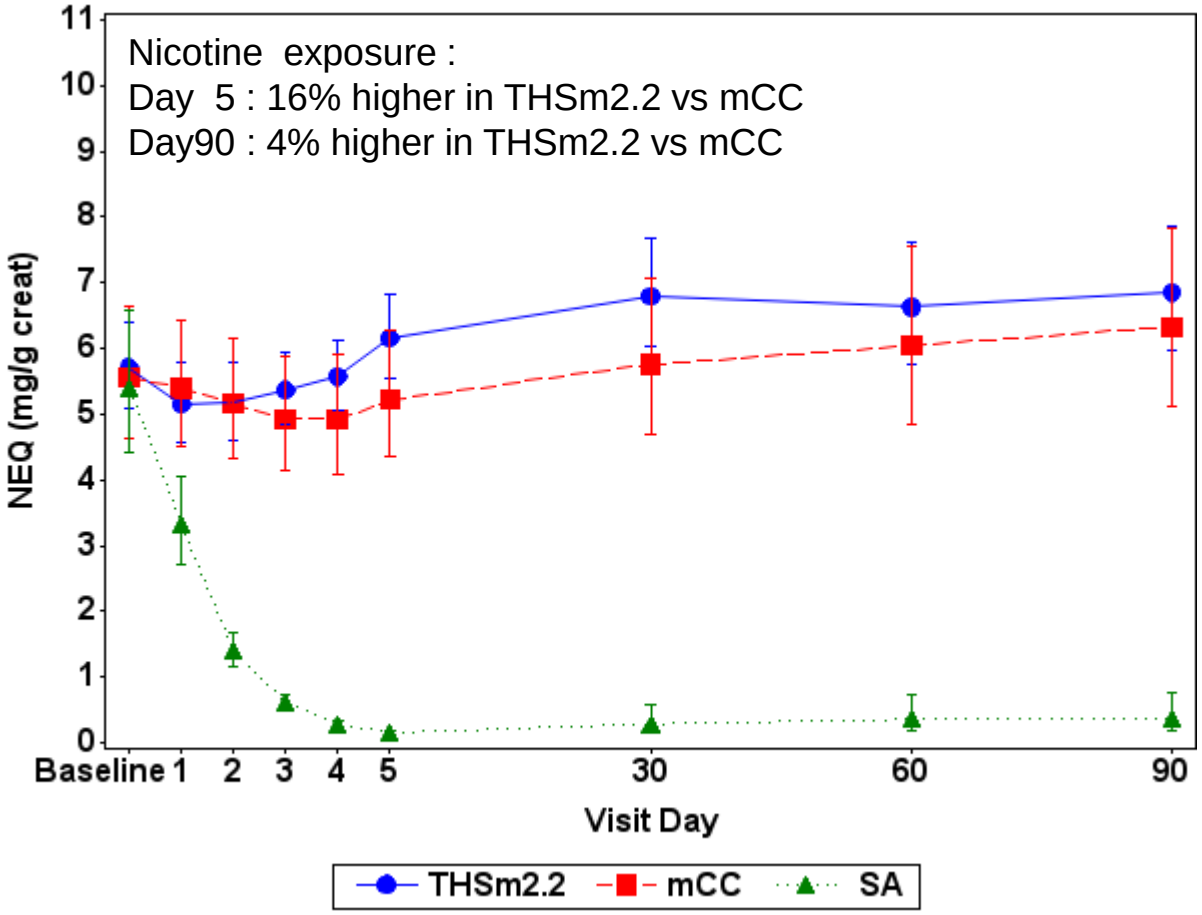
Nicotine Uptake



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

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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

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Safety

- No serious or severe adverse events (AE).
- 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, unexpected AE related to product in THS arm (diarrhea). Most frequent AEs were decreased hemoglobin and neutrophils.
- No clinically relevant abnormality in vital signs, ECGs, or physical examination.

Publications for the clinical study

- Lüdicke F, Picavet P, Baker G, Haziza C, Poux V, Lama N, Weitkunat R. Effects of Switching to the Tobacco Heating System 2.2 Menthol, Smoking Abstinence, or Continued Cigarette Smoking on Biomarkers of Exposure: A Randomized, Controlled, Open-Label, Multicenter Study in Sequential Confinement and Ambulatory Settings (Part 1). Nicotine Tob Res. 2018 Jan 5;20(2):161-172.
- Lüdicke F, Picavet P, Baker G, Haziza C, Poux V, Lama N, Weitkunat R. Effects of Switching to the Menthol Tobacco Heating System 2.2, Smoking Abstinence, or Continued Cigarette Smoking on Clinically Relevant Risk Markers: A Randomized, Controlled, Open-Label, Multicenter Study in Sequential Confinement and Ambulatory Settings (Part 2). Nicotine Tob Res. 2018 Jan 5;20(2):173-182.

Common conclusion

- Both studies demonstrated that switching from mC 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, THS 2.2 use is associated with favorable shifts in the direction of SA. Similarly, in ApoE$^{-/-}$ mice, switching from cigarette smoke exposure to THS 2.2 aerosol exposure reverses the onset of disease in a similar manner as cessation.

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Thank you for your attention