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Pre-clinical and clinical studies in COPD and Cardiovascular areas and the Role of Biomarkers

James Battey

This research was funded by Philip Morris International

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 (RRPs) so that consumer acceptance can be best fulfilled

*who want to continue using nicotine products

**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 RRP's in various stages of development, scientific assessment and commercialization.

Because our RRP's 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DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.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be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance.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For questions regarding this draft guidance, contact the Center for Tobacco Products at (Tel) 1-877-CTP-1373 (1-877-287-1373) Monday-Friday, 9:00 a.m. – 4:00 p.m. EDT.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

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

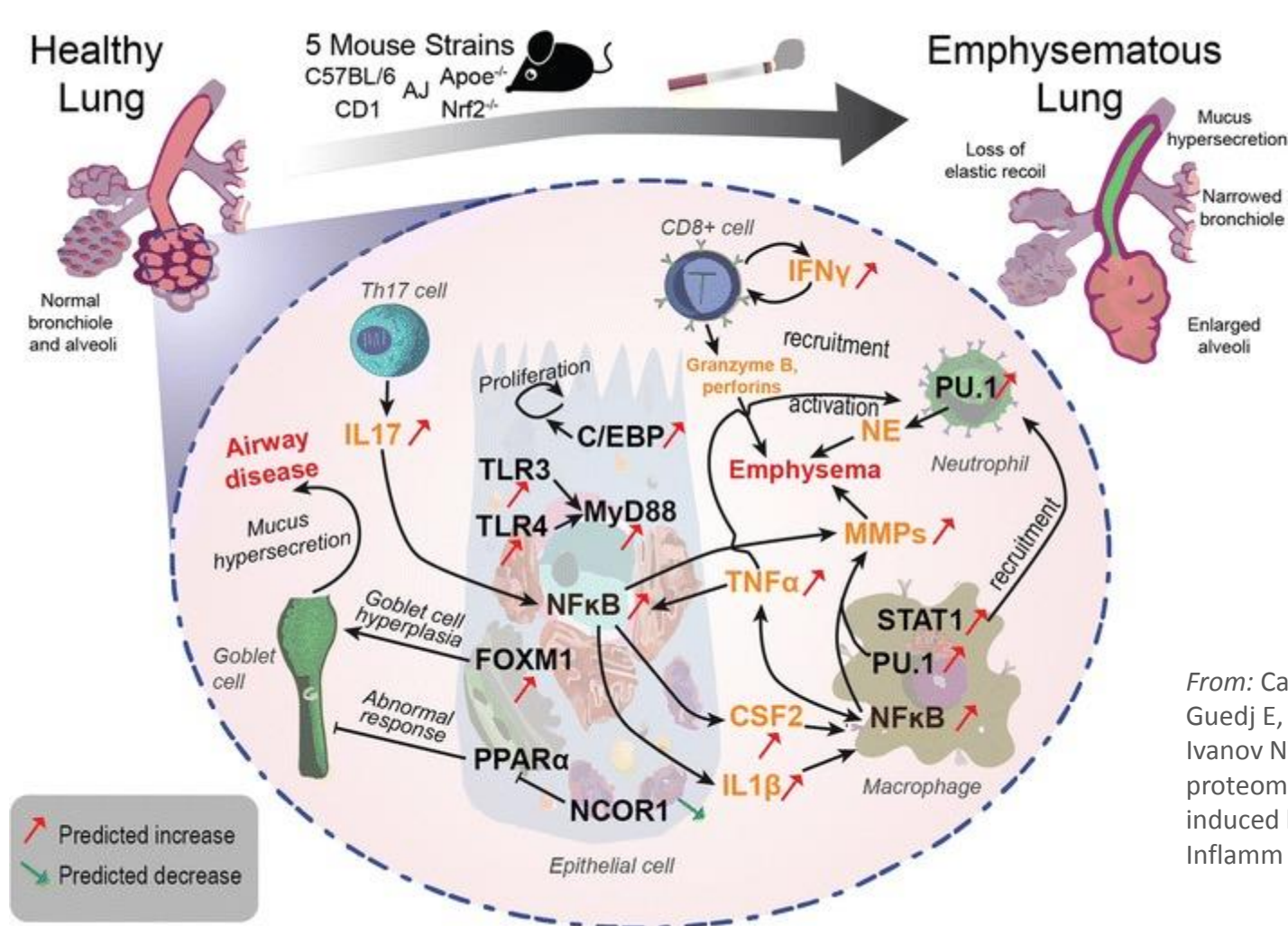
Aim and scope of the presentation

- Assessment of the effects of conventional cigarette smoke and RRP, using PMI's Heat-not-Burn technology, in 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

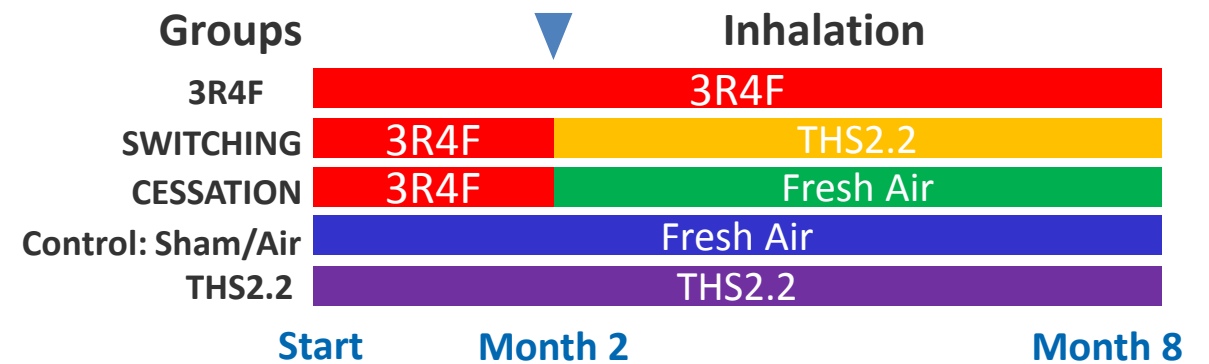
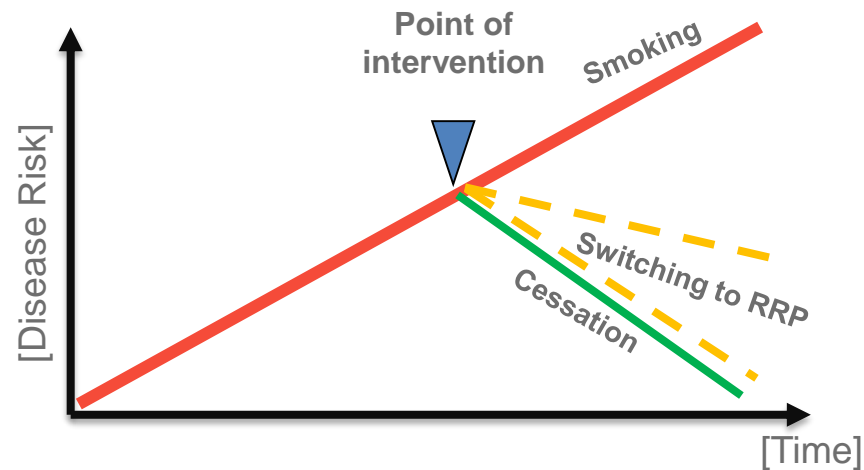
Potentially Reduced-Risk product:
Aerosol from Heatsticks and
Tobacco Heating System, THS 2.2



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

ApoE^{-/-} mouse switching study

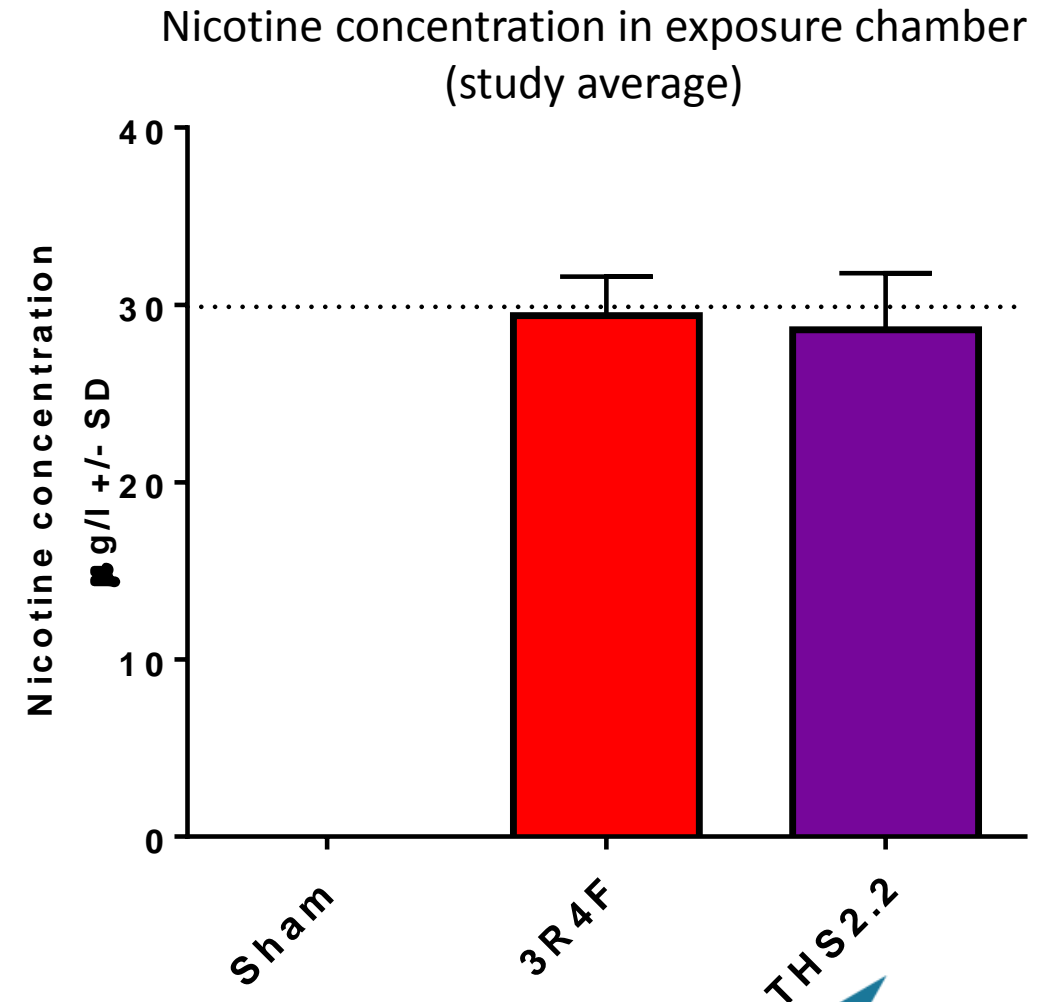
Methods - Exposure regime



 Fresh air

 THS2.2 or 3R4F

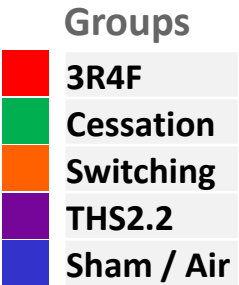
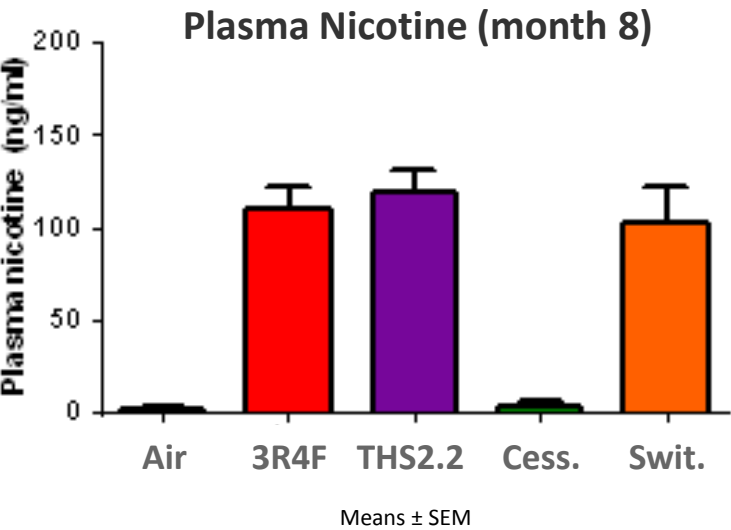
- Animals were exposed 3 hours per day (3 x 1 hour interrupted exposure periods), 5 days per week
- Nicotine was measured during every exposure period (3 samples per chamber per day)
- Aerosol delivery (nicotine) was within +/- 10% of the targeted **29.9 µg/l** nicotine concentration



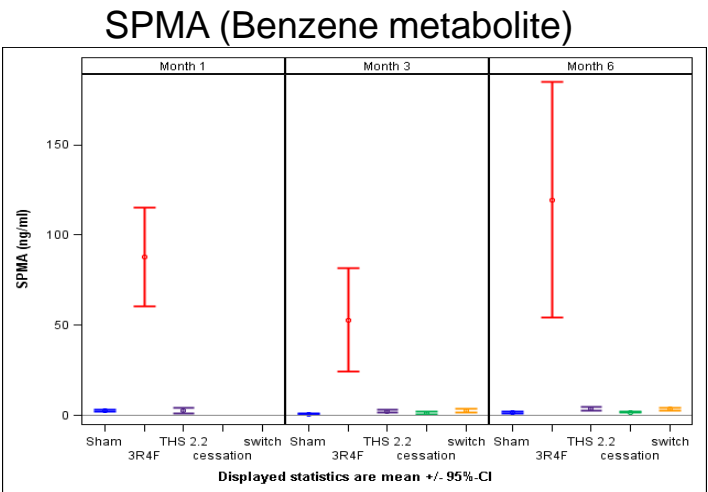
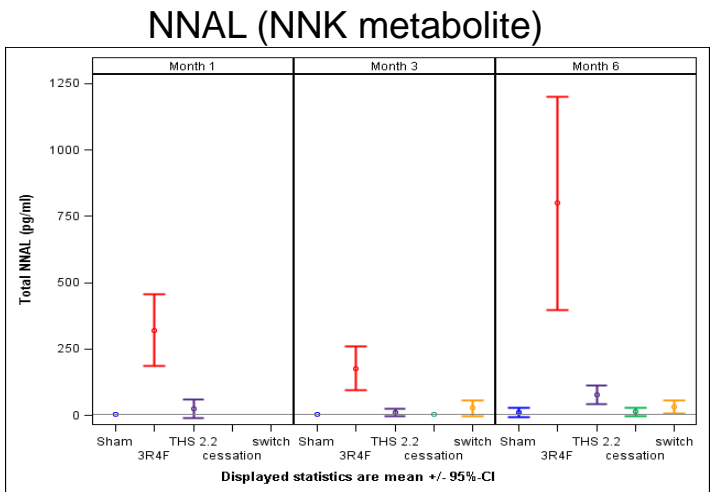
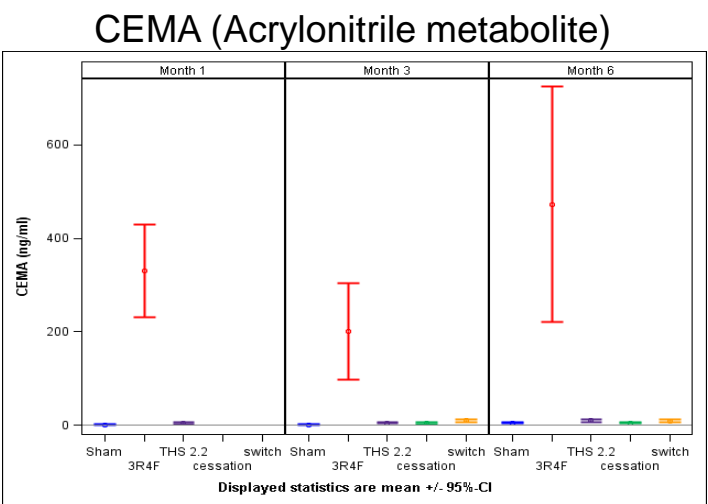
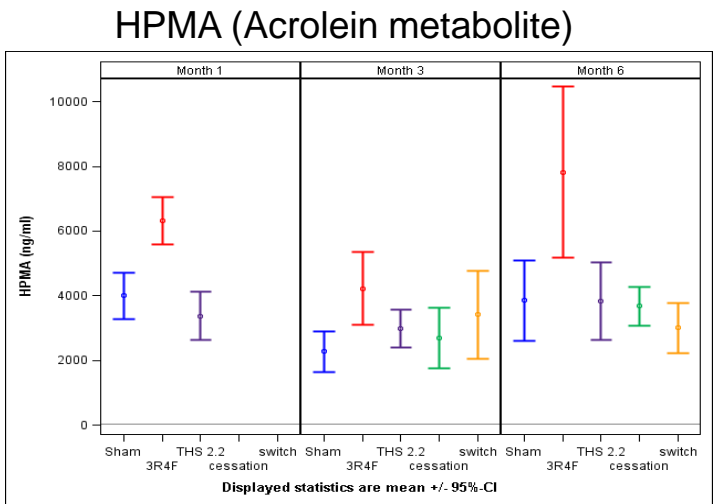
*29.9 µg/l nic corresponds to 6.5 mg/kg, daily dose- or the nicotine amount from approx. 32 cig/day for a 60 kg human, based on body surface comparison, Guidance document Heq dose, FDA

ApoE^{-/-} mouse switching study

Aerosol uptake (biomarkers of exposure)



Urinary Metabolites (months 3, 6, 8)

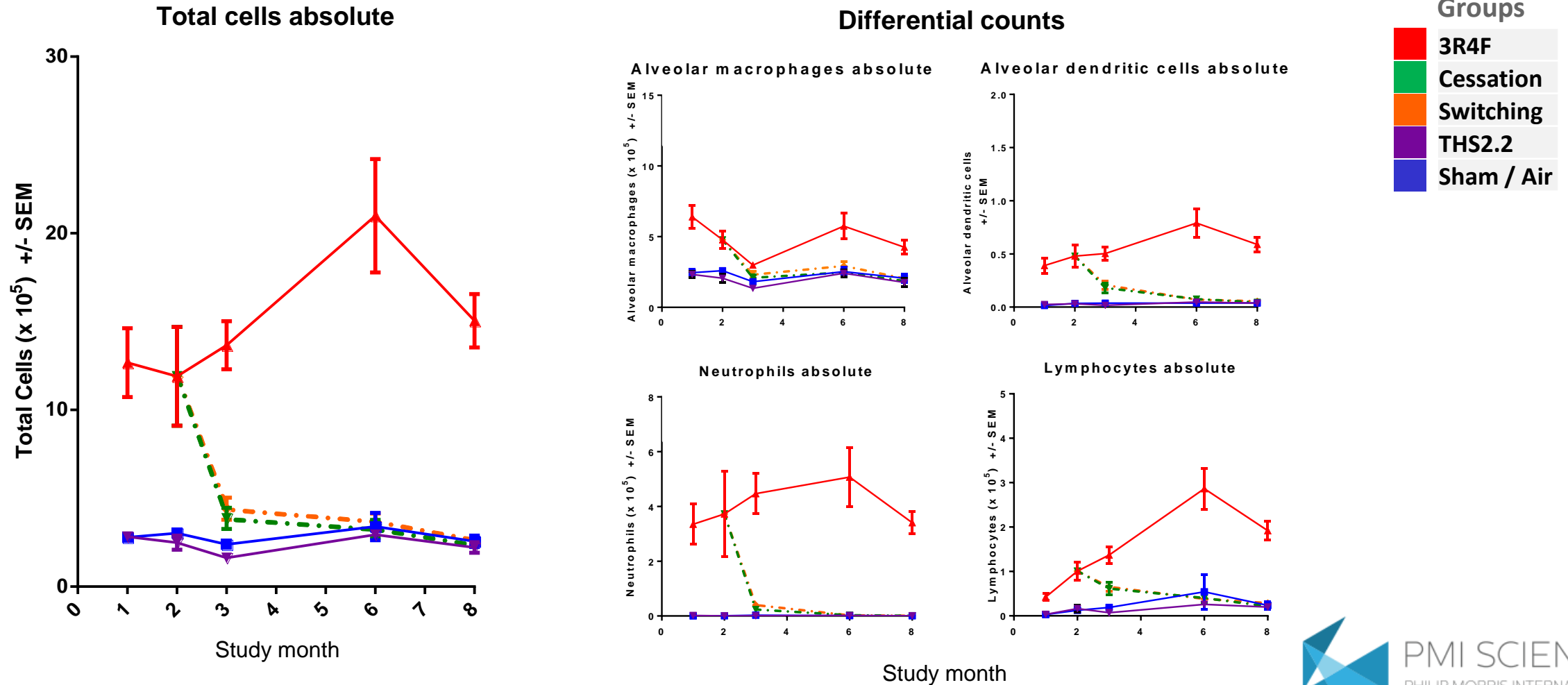


Means ± SEM

ApoE^{-/-} mouse switching study

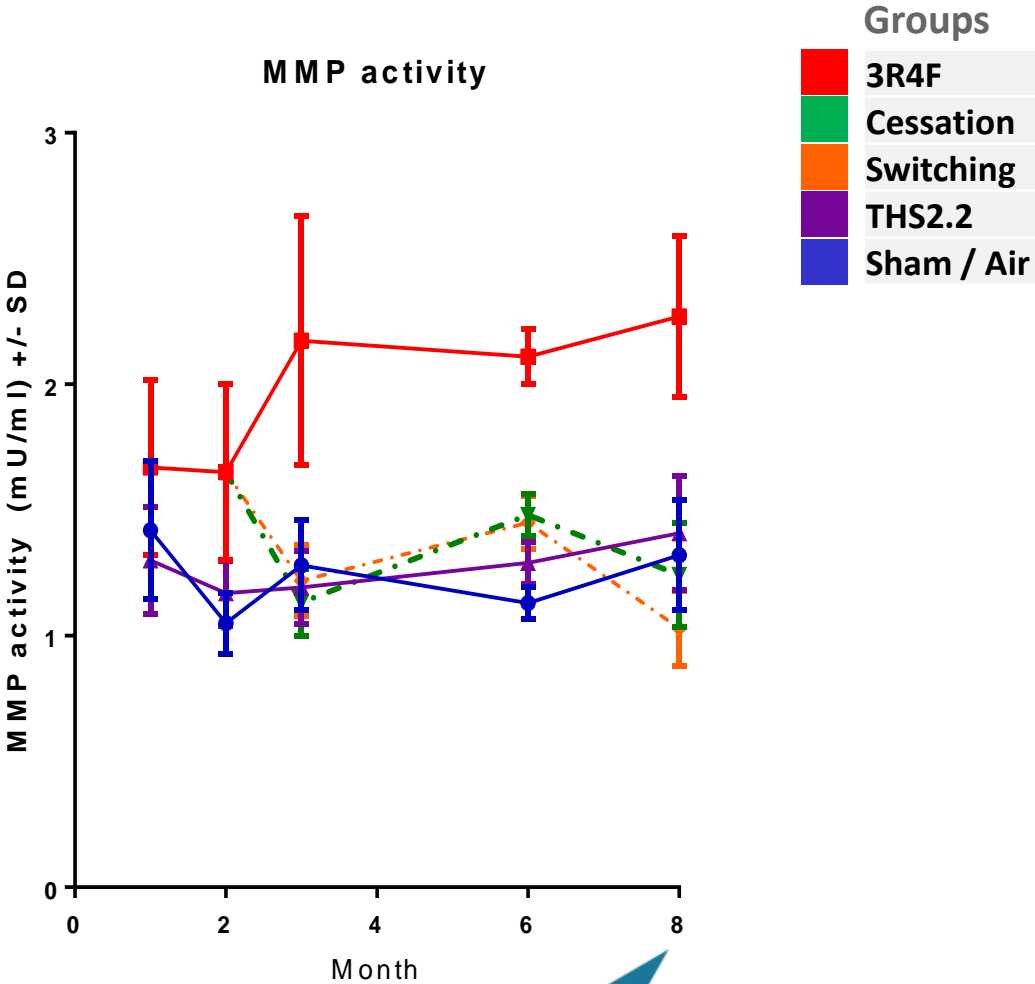
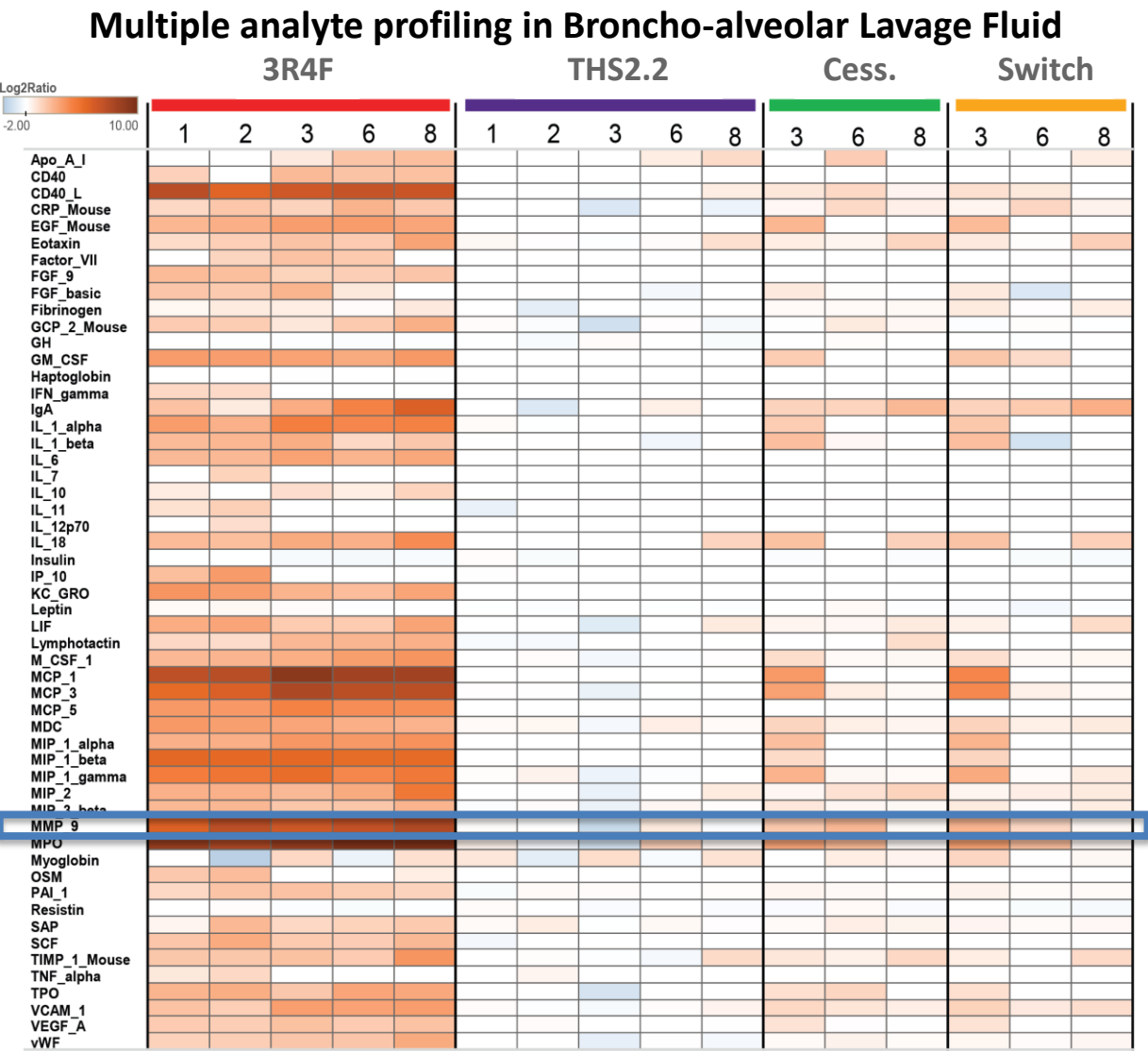
Result summary: Disease mechanisms - Lung inflammation

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



ApoE^{-/-} mouse switching study

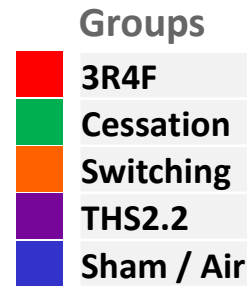
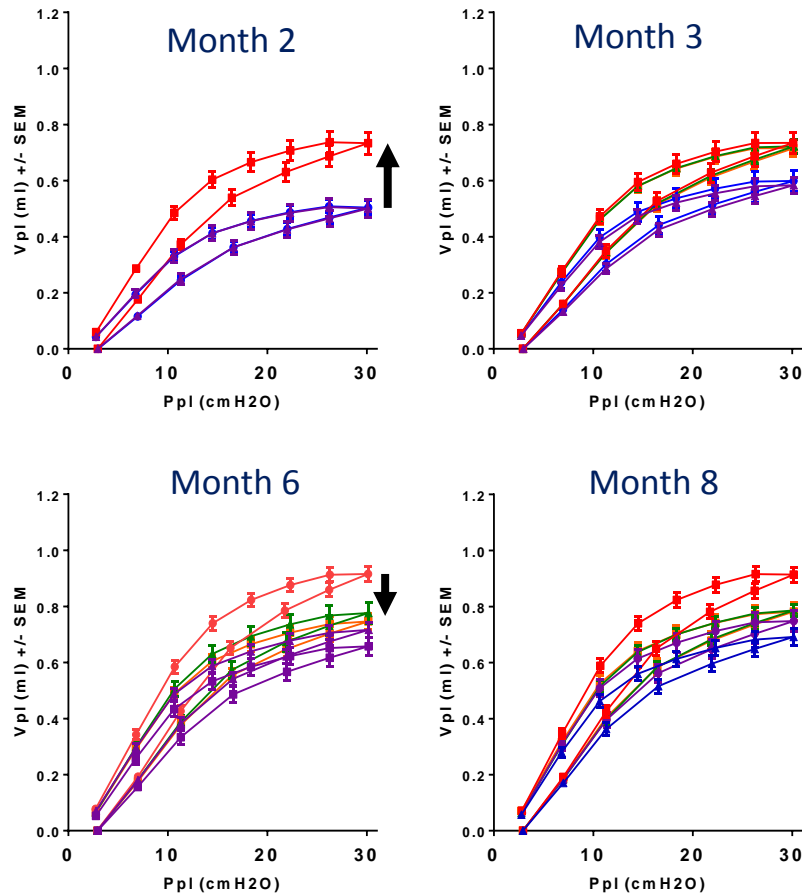
Result summary: Disease mechanisms - Lung inflammation



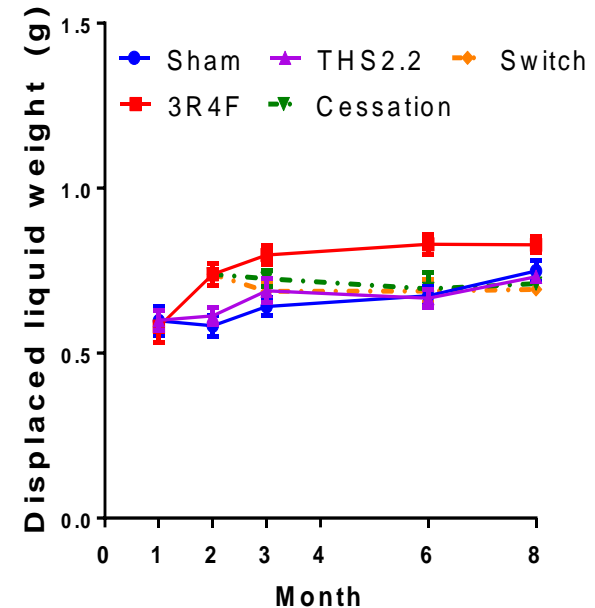
ApoE^{-/-} mouse switching study

Result summary: Disease endpoints- Lung function and lung volume

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



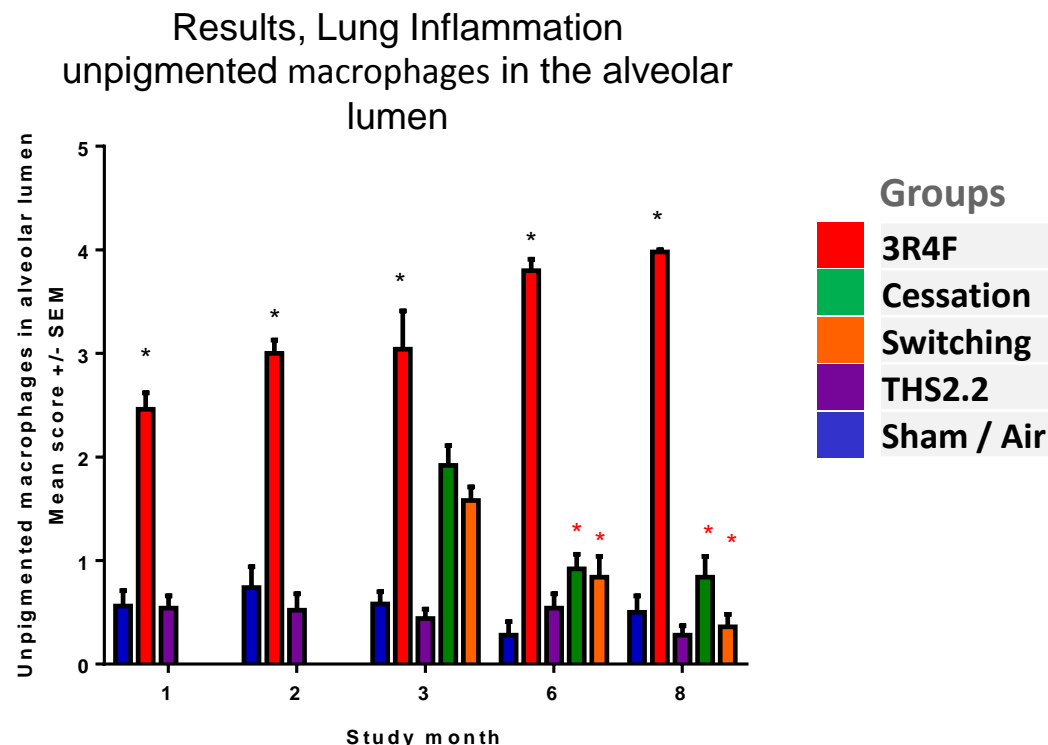
Lung volume (absolute, measured)



Means \pm SEM

ApoE^{-/-} mouse switching study

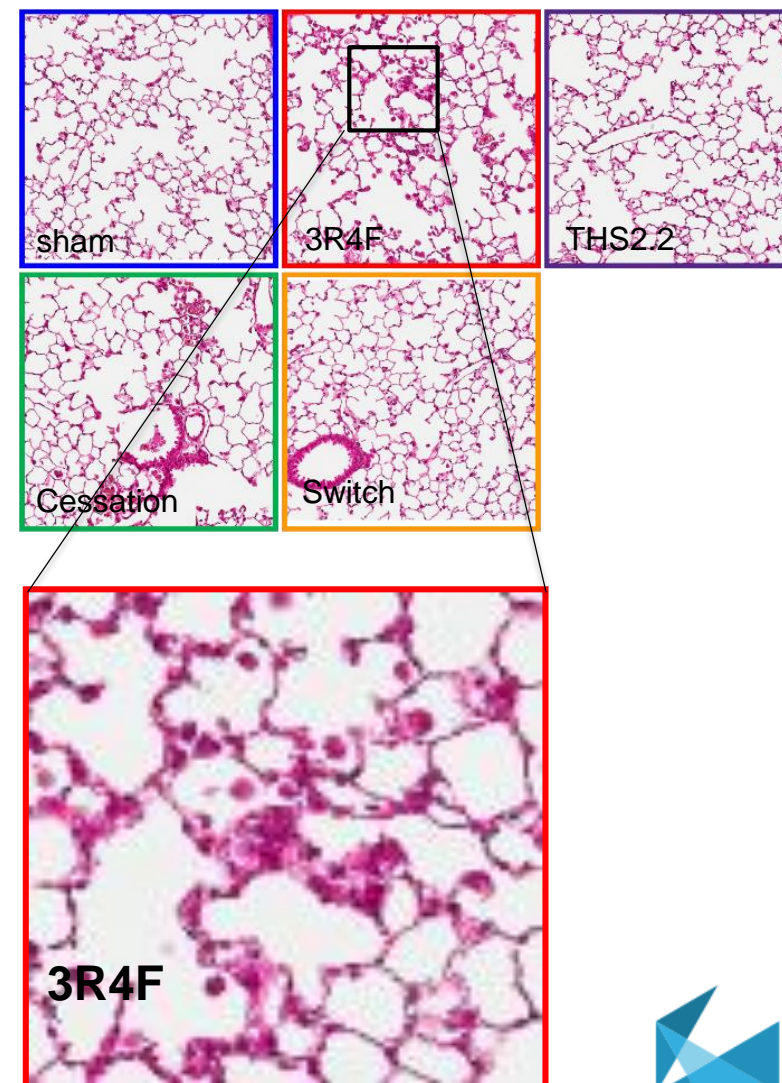
Result summary: Histopathology of the lung - Pulmonary inflammation



*: Statistically significant compared to sham

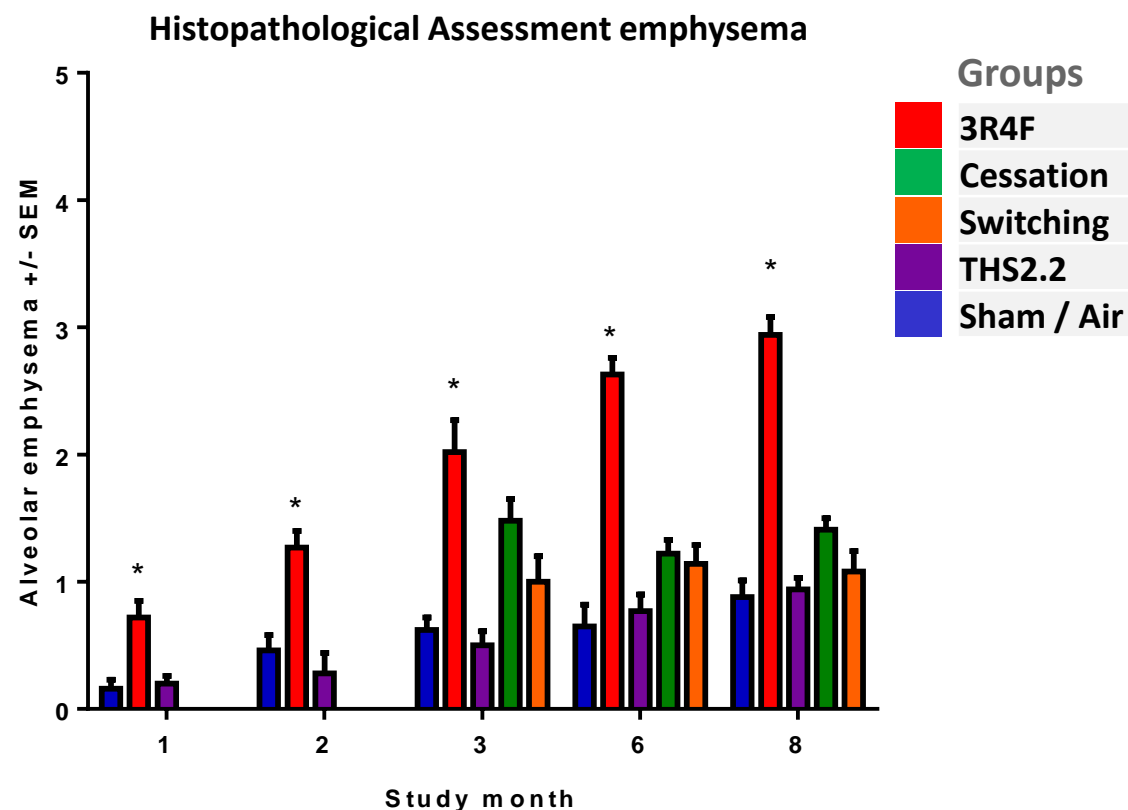
*: Statistically significant compared to 3R4F:CONT at month 2

- 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



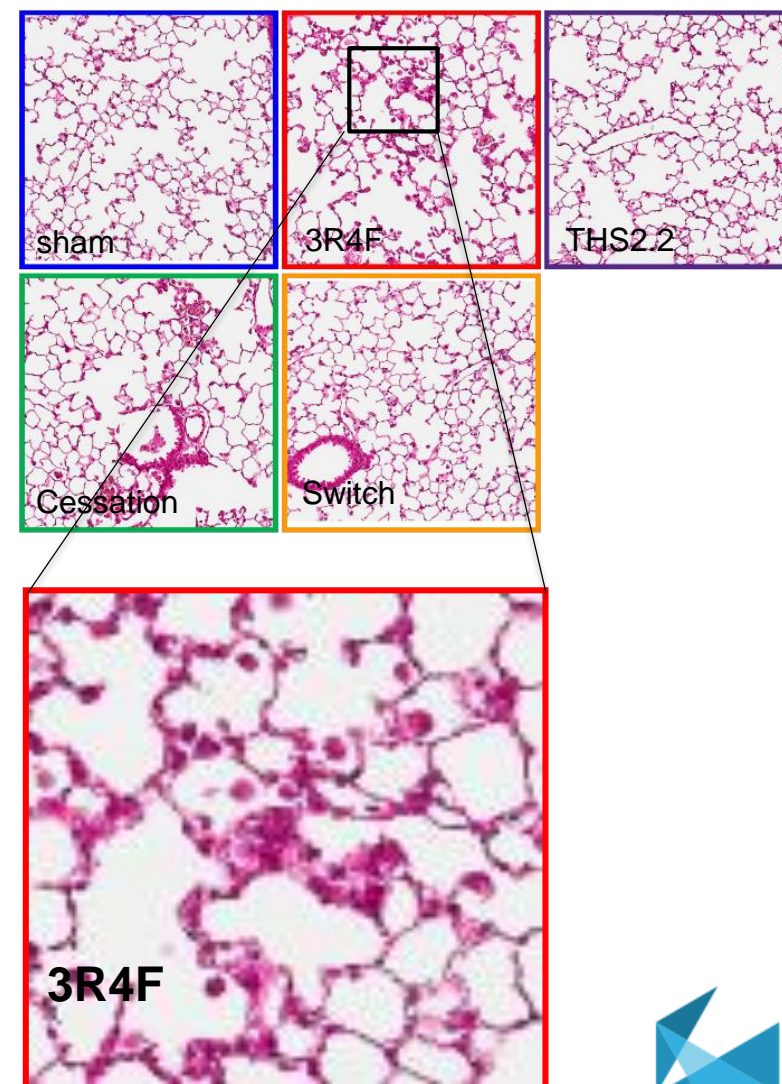
Switching Study in an Animal Model of Disease

Result summary: Tissue changes - Histopathology



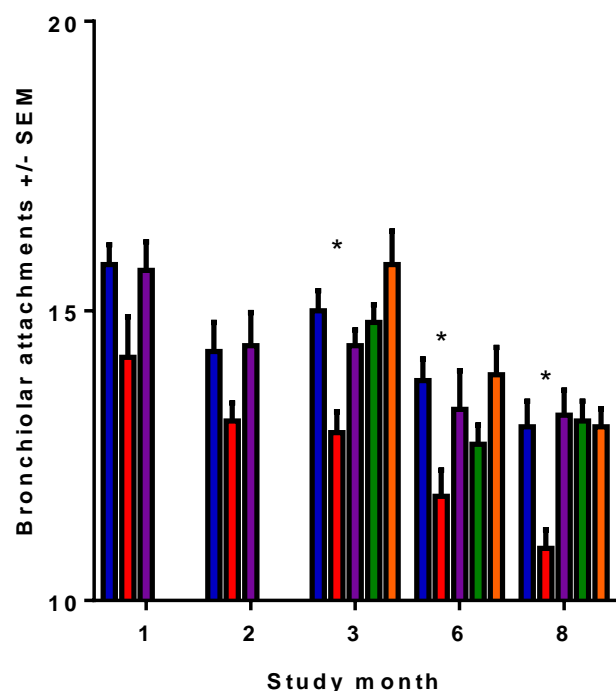
*: Statistically significant compared to sham

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



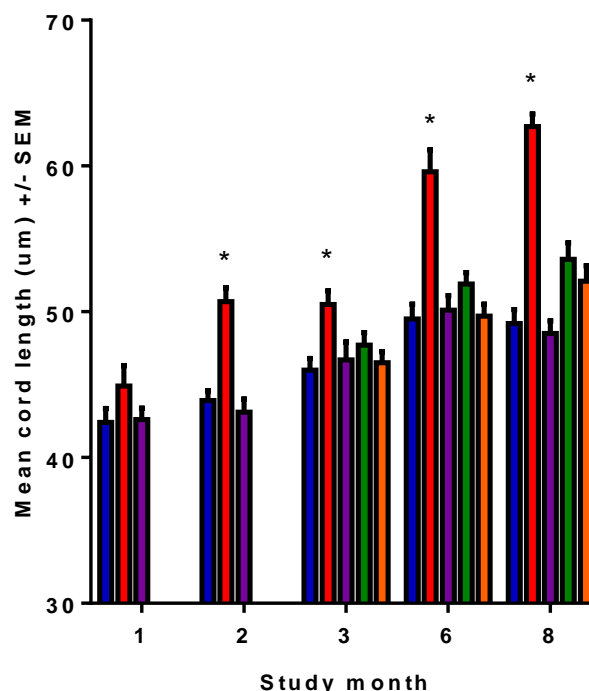
ApoE^{-/-} mouse switching study

Result Summary: Lung tissue changes - Morphometry



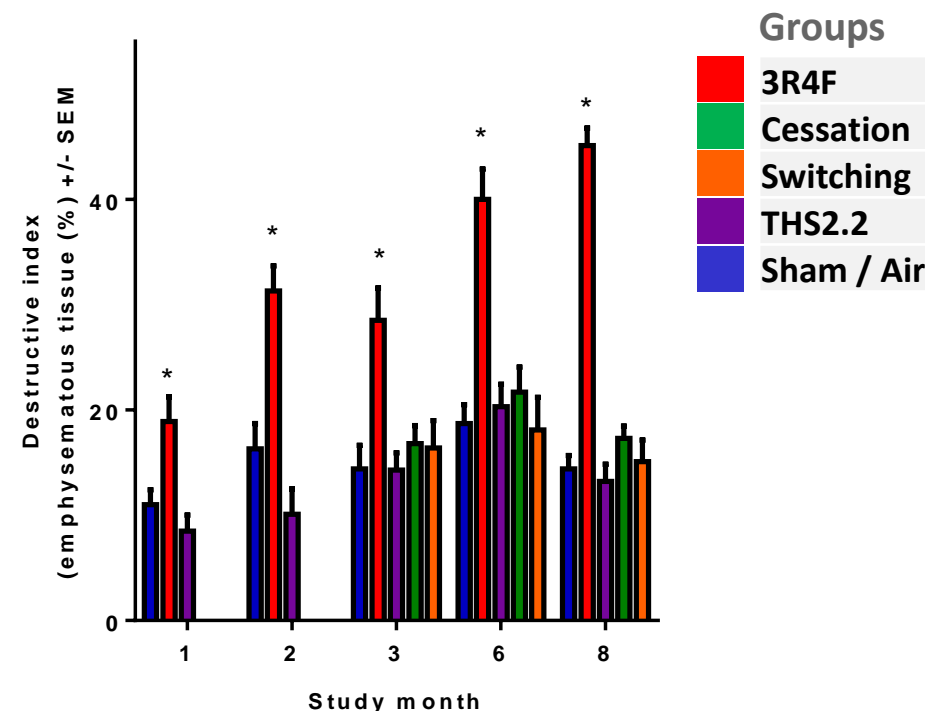
Bronchiolar attachments

- Fewer Bronchiolar attachments in 3R4F-exposed group



Mean chord length (MCL)

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



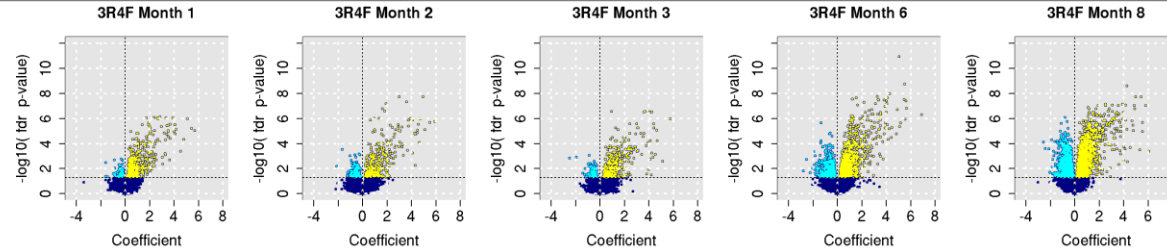
Destructive index (DI)

- Index of parenchymal destruction
- Increased DI in 3R4F-exposed group

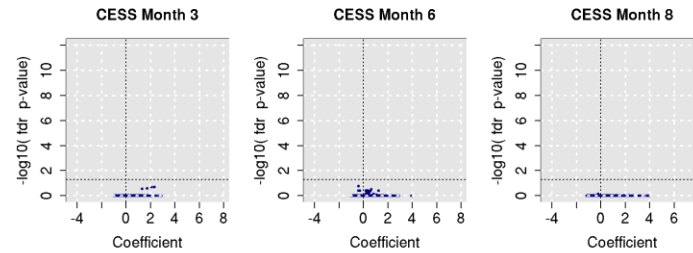
ApoE^{-/-} mouse switching study

Result Summary: Systems response profile: differential gene expression - Lung

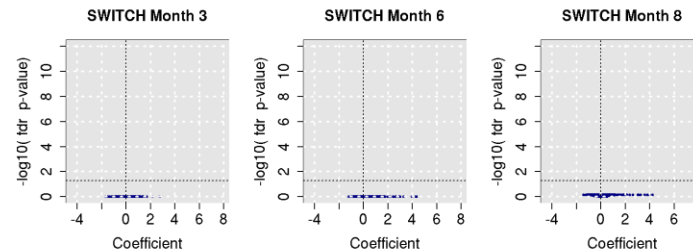
3R4F



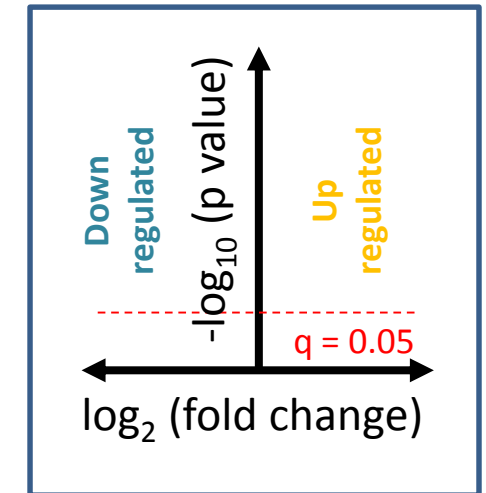
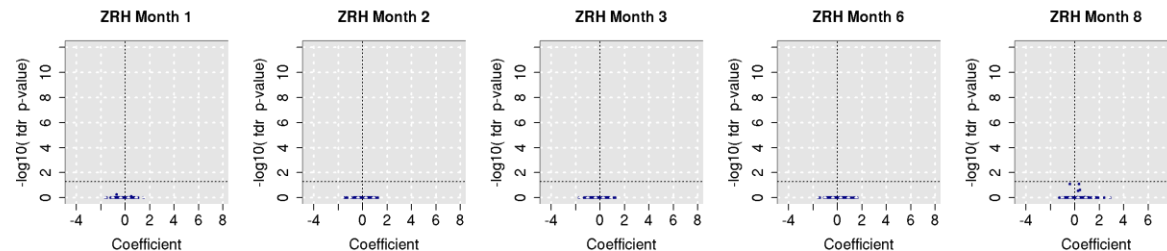
Cessation



Switch

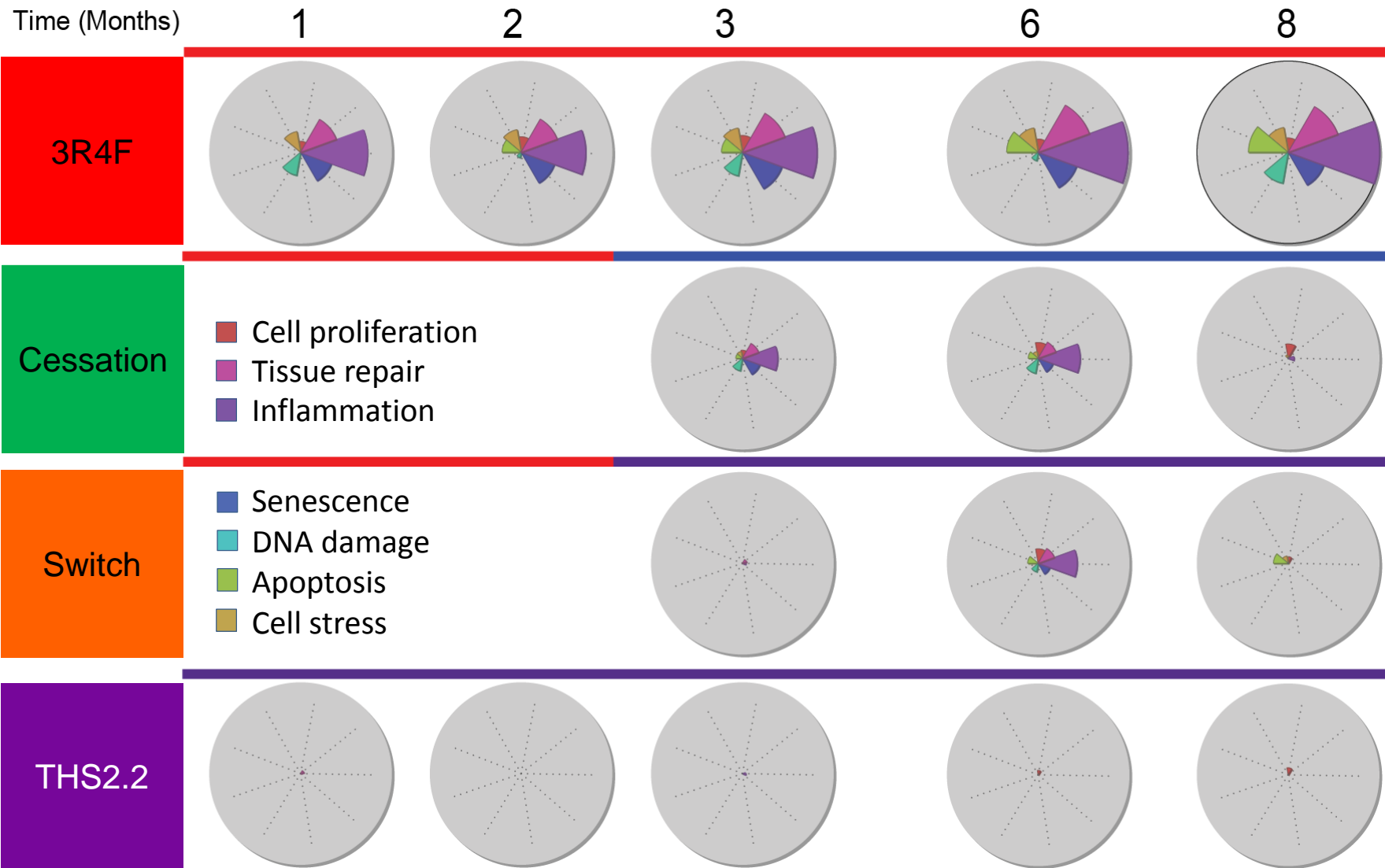


THS2.2



ApoE^{-/-} mouse switching study

Result Summary: Disease mechanisms - Network perturbations - Lung



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 Cigarettes. *Toxicol 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 adult 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

Study Products and Interventions



= THSm2.2
(Tobacco Heating System 2.2 menthol)



= SA
(Smoking abstinence)



= mCC
(Menthol conventional cigarettes)

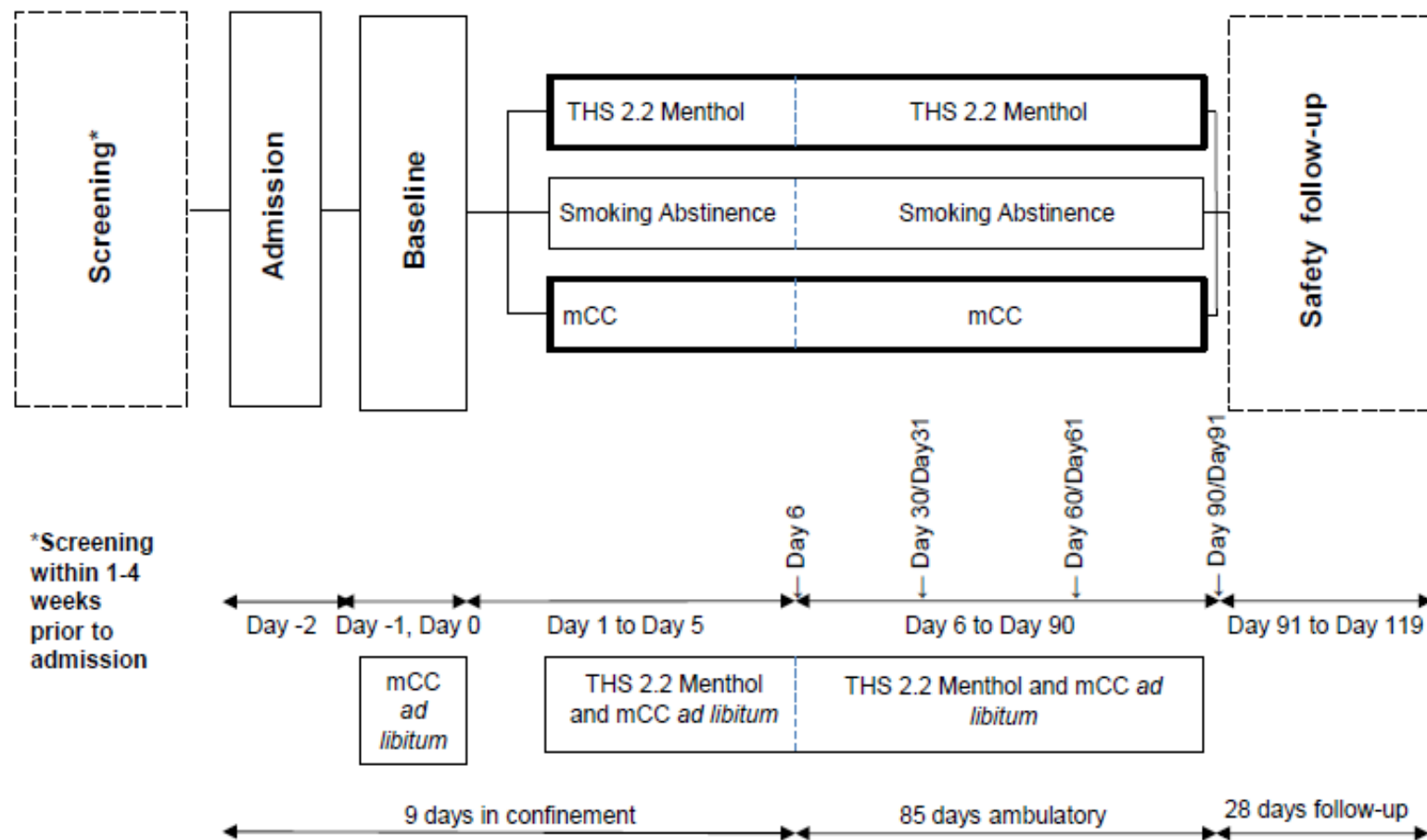
Primary Objective and Endpoints

- To demonstrate the reduction of biomarkers of exposure (BoExp) to harmful and potentially harmful constituents (HPHCs) in adult smokers switching from menthol conventional cigarette (mCC) to THS 2.2 Menthol (THSm2.2) compared to adult 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

Study Design



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



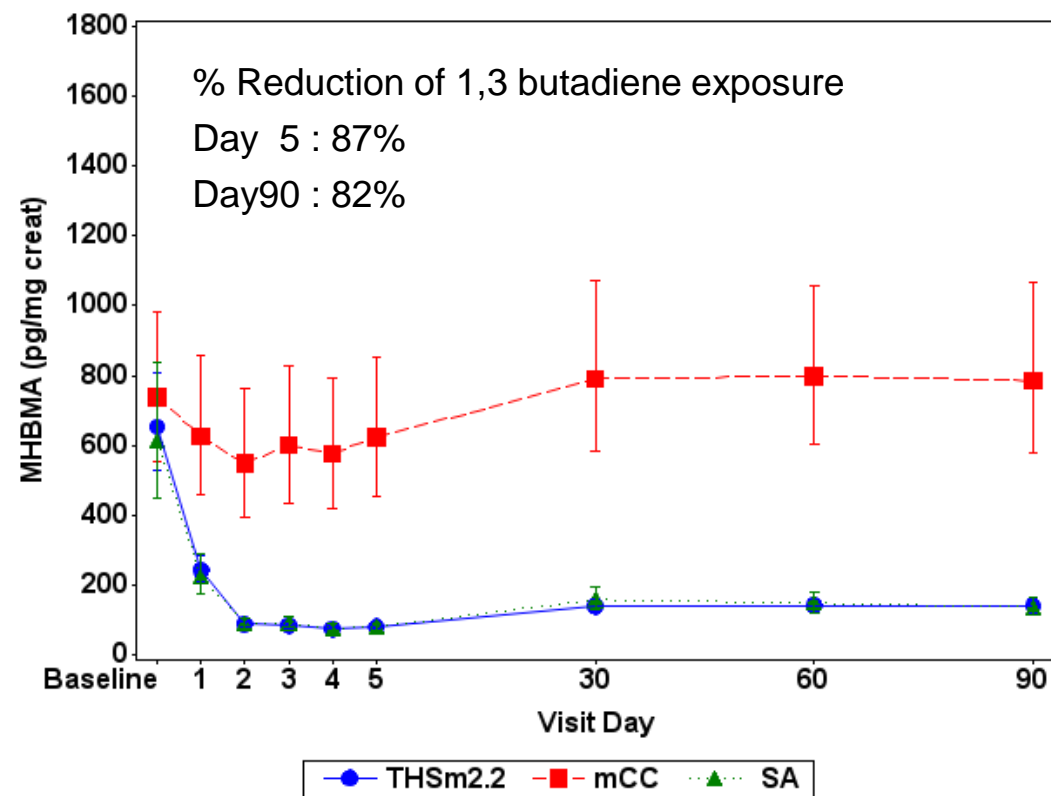
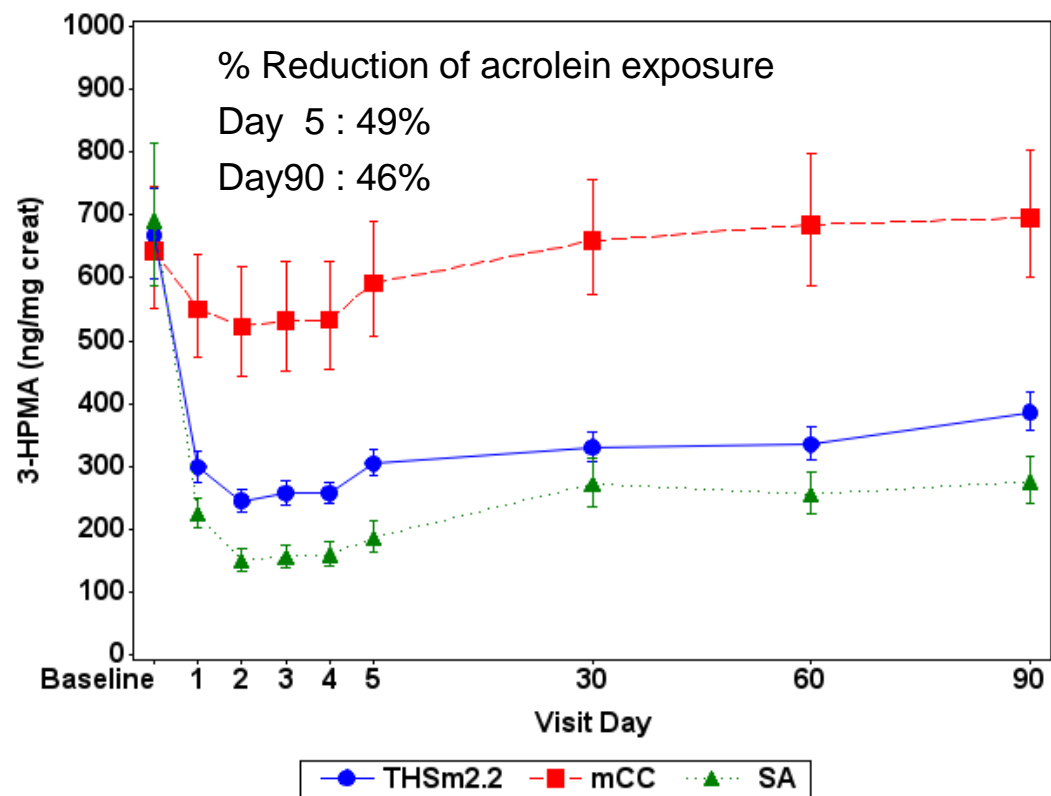
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Japanese Population Characteristics

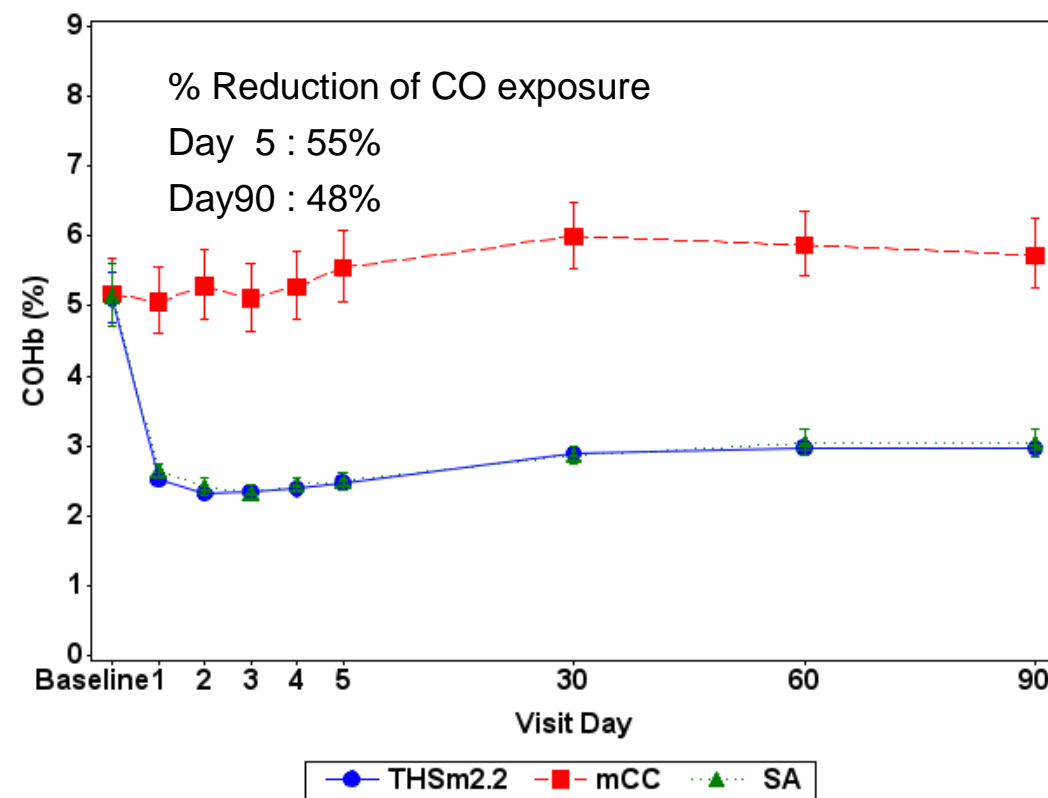
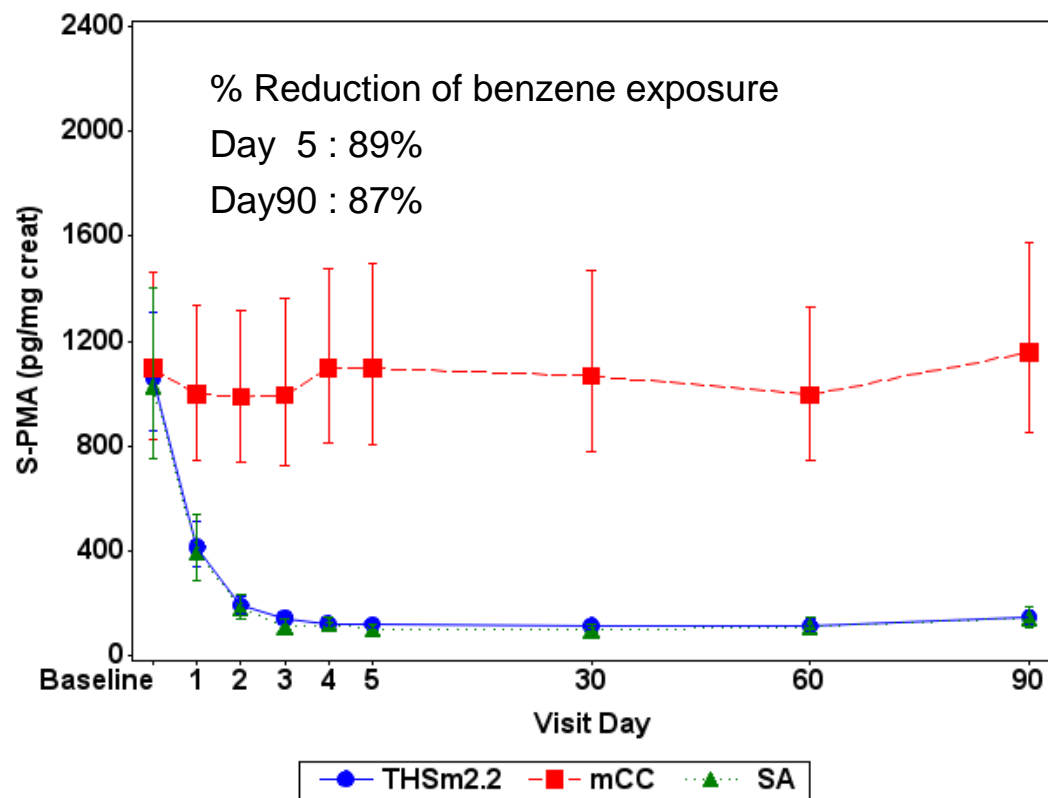
Characteristics	THSm2.2 (N=78)	mCC (N=42)	SA (N=40)	Overall (N=160)
Females – n(%)	33 (42.3)	17 (40.5)	18 (45.0)	68 (42.5)
Age (years) - Mean±SD	37 ± 11	37 ± 11	37 ± 10	37 ± 11
BMI Normal Weight– n(%)	60 (76.9)	32 (76.2)	32 (80.0)	124 (77.5)
Daily mCC Consumption– n(%)				
10-19 cig/day	40 (51.3)	23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

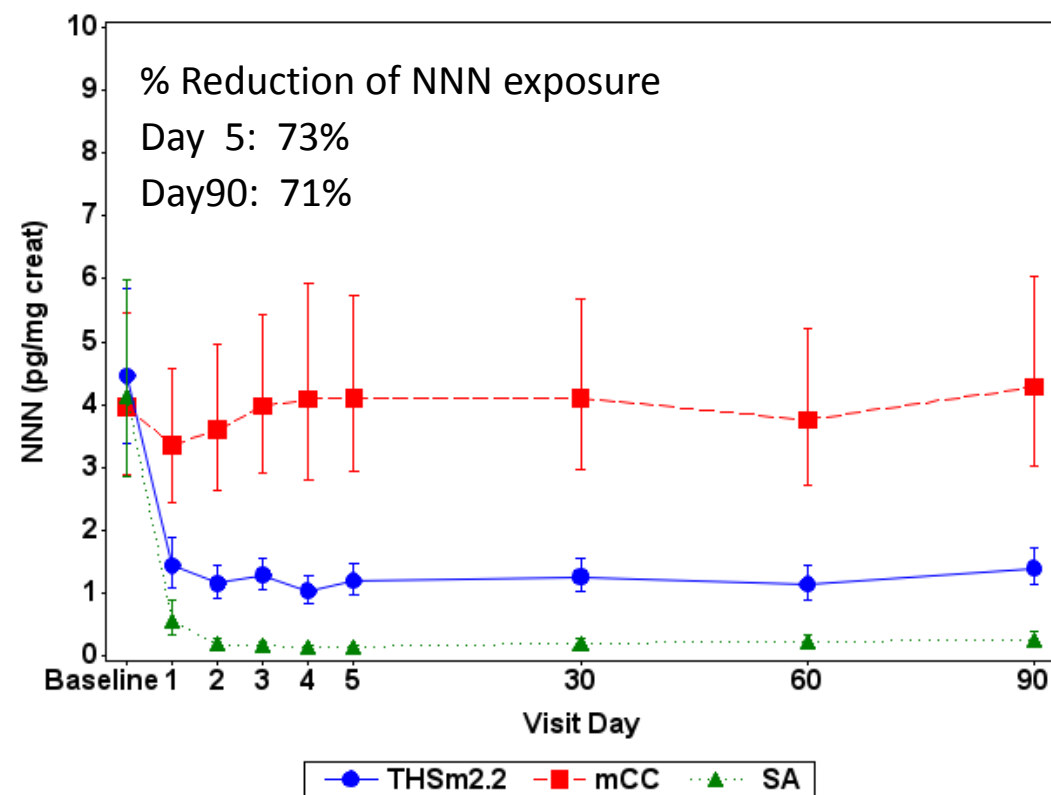
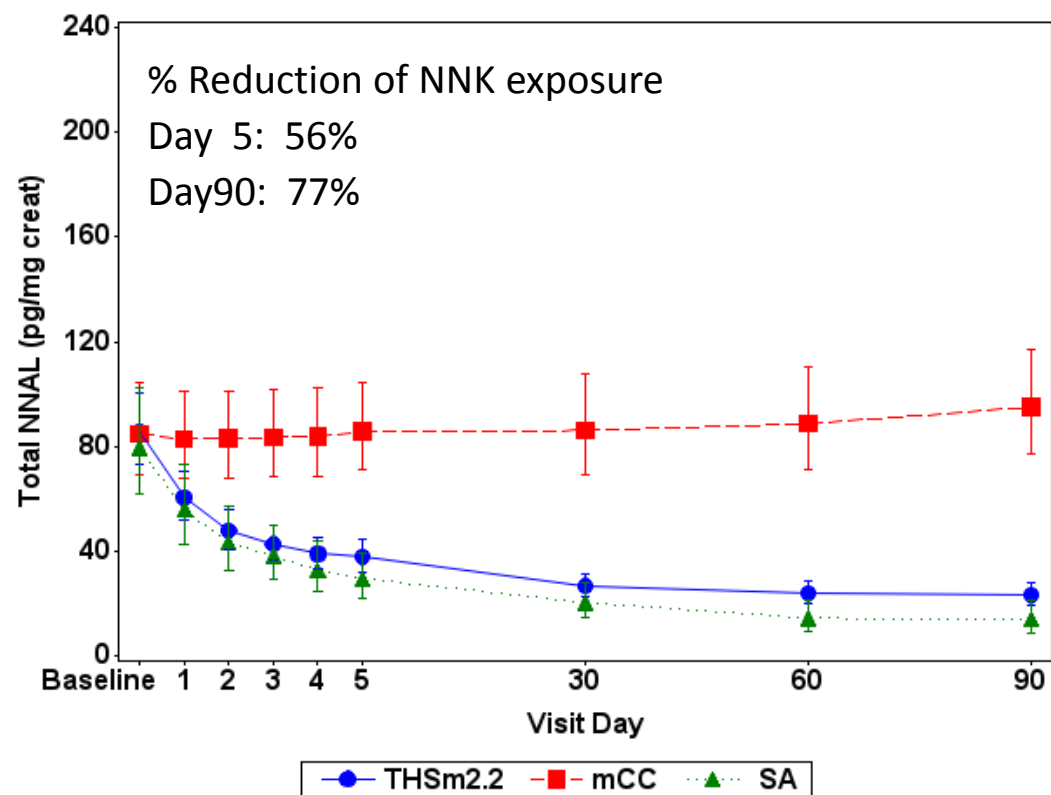
Biomarkers following 90 days of exposure (1/3)



Biomarkers following 90 days of exposure (2/3)



Biomarkers following 90 days of exposure (3/3)



Product Use – Average Daily Product Use

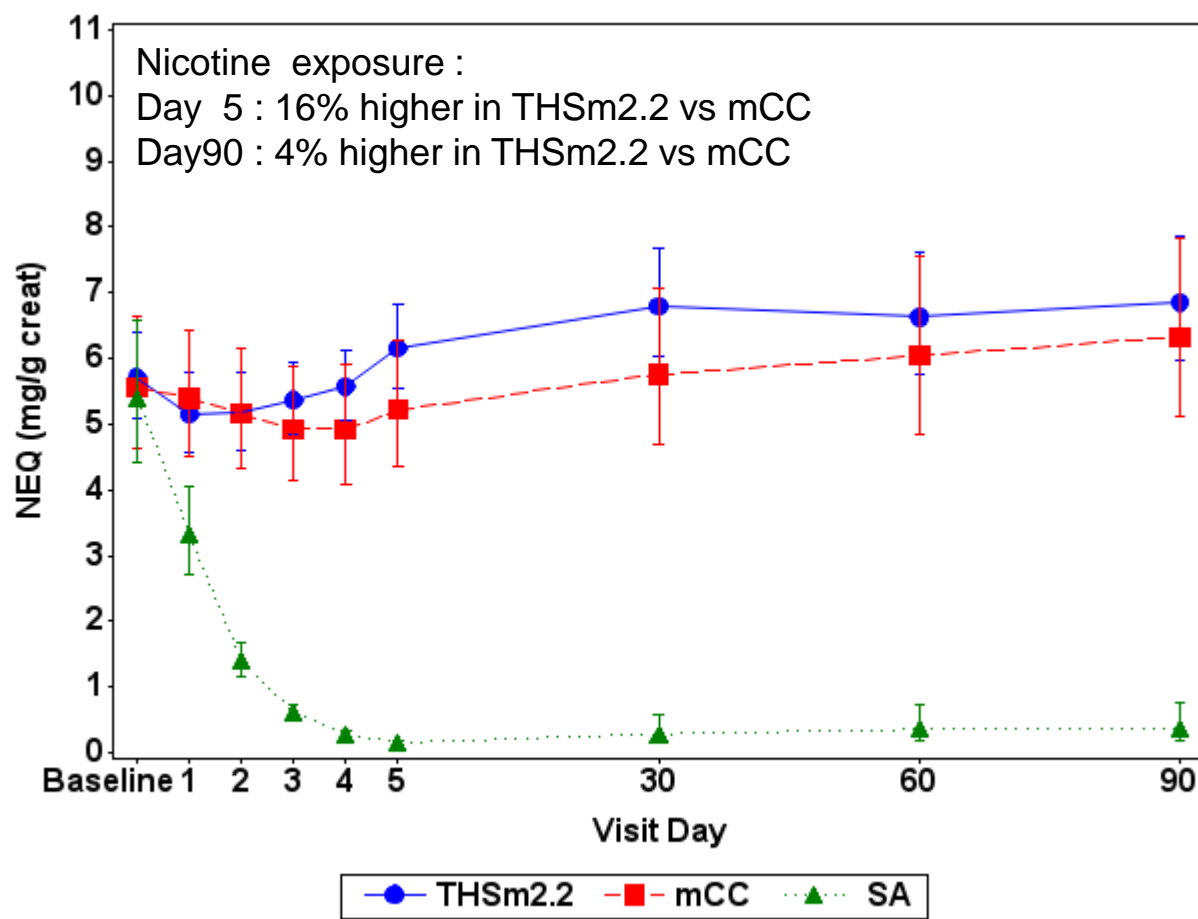
Time point	THSm2.2 Mean±SD	mCC Mean±SD
Baseline mCC	13.1±3.8	12.5±3.9
Day 5	13.9±4.3	13.6±4.7
Day 5-30	11.7±5.9	13.8±4.2
Day 30-60	12.7±6.2	14.9±5.7
Day 60-90	12.7±6.5	15.2±5.0

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

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 _{2α}	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.

Common conclusion

- Both 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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Thank you for your attention
