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

Dr. Julia Hoeng, PhD
Director Systems Toxicology

PMI R&D
Philip Morris Products S.A.
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CH-2000 Neuchâtel, Switzerland
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.

* 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

- 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

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

**Epidemiology**

**Sequence of events leading to smoking-related diseases**

1. **Chronic Cigarette Smoke Exposure**
2. **Molecular Changes**
3. **Disruption of Biological Mechanism**
4. **Cell/Tissue Changes**
5. **Physiological Changes**
6. **Disease (CVD, COPD, Lung Cancer)**
7. **Population Harm**

**Analytical Chemistry**

**Biological Networks – Systems Biology/Toxicology**

**Medicine**

**Public Health**

**Aerosol Chemistry and Physics**

**Standard/Systems Toxicology Assessment**

**Clinical Trials**

**Post-Market Studies & Surveillance**

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

Lower temperatures reduce constituents in the aerosol
Nicotine is transferred via distillation
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
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.
- 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
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<sup>−/−</sup> mice are the most widely used pre-clinical model of atherosclerosis.

ApoE<sup>−/−</sup> mice show delayed lipoprotein clearance and consequently develop hyper- and dyslipoproteinemia, severe hypercholesterolemia, and atherosclerotic lesions, even when on a normal diet.


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?

Point of intervention

[Diagram showing progression of disease risk over time with interventions like switching to THS 2.2 and cessation compared to ongoing smoking.]
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.

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

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

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

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?

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

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

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

ApoE/− mice respiratory system

Lung cast

ApoE−/− mice respiratory system

~ 1 cm

Study 2 (2018)
Body weight progression

Biomarkers of exposure

Example images of stained plaque in aorta

Percentage of plaque based on mm²

Time (months)  | 1  | 2  | 3  | 6  | 8  |
---|---|---|---|---|---|
Mean ± SEM    |   |   |   |   |   |

Exposure group
- Sham
- 3R4F
- THS 2.2
- Cess
- Switch

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

<table>
<thead>
<tr>
<th>Glycerophospholipids</th>
<th>3R4F</th>
<th>THS2.2</th>
<th>8m</th>
<th>Cessation</th>
<th>Switch</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPC 16:0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPC 18:0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPC 18:2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC 16:0/22:5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC 18:0/20:3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE 18:0/20:3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Sphingolipids        |      |        |    |           |        |
| SM (d18:1/15:0) (d18:1/14:0) | |        |    |           |        |

| Sterol Lipids        |      |        |    |           |        |
| CE 14:0              |      |        |    |           |        |
| CE 16:1              |      |        |    |           |        |
| CE 18:1              |      |        |    |           |        |
| CE 18:3              |      |        |    |           |        |
| CE 22:3              |      |        |    |           |        |
| CE 22:4              |      |        |    |           |        |
| CE 22:5              |      |        |    |           |        |
| CE 22:6              |      |        |    |           |        |

“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)
Sham
Reference cigarette
THS 2.2
Cessation
Switch

Morphometric analysis indicates CS-induced emphysema

Differentially expressed genes (all vs. sham)

Myc, Cox-2, Il1, Fos, Tgfb, Myd88, Jun, Mxi, Il1, Myb, Stat, Jak, Myc, Cox-2, Il1, Fos, Tgfb,

I’ll just check my favorite gene.
Mechanisms Impacted in the Lung

**Lung Inflammation**

- **Total lung cells**
  - Sham
  - 3R4F
  - THS 2.2

- **Cessation**
  - Switch

**Mediators in BALF**

**Cardiovascular**

- **Micro CT**

**Lung function**

- **Month 1**
  - Ppl (cm H$_2$O) $\pm$ SEM

- **Month 2**
  - Ppl (cm H$_2$O) $\pm$ SEM

- **Month 3**
  - Ppl (cm H$_2$O) $\pm$ SEM

- **Month 6**
  - Ppl (cm H$_2$O) $\pm$ SEM

**Summary of effect versus 3R4F**

<table>
<thead>
<tr>
<th>Biomarkers/Endpoints</th>
<th>THS 2.2</th>
<th>Cessation</th>
<th>Switching to THS 2.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NNAL, 3-HPMA, SPMA, CEMA, COHb</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
</tr>
<tr>
<td>Rate of atherosclerotic plaque growth</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
</tr>
<tr>
<td>Blood lipids (including 11-OHTX-82 and isoprostanes)</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
</tr>
<tr>
<td>Aorta lipidomics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung function measured using a FlexVent system</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
</tr>
<tr>
<td>Histopathological evaluation of the respiratory nasal epithelium</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
</tr>
<tr>
<td>Histopathological evaluation of the lung tissue</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
</tr>
<tr>
<td>Inflammatory mediators and cells in the bronchoalveolar lavage fluid (BALF)</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
</tr>
<tr>
<td>Lung lipidomics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole transcriptome analysis of the Respiratory Nasal Epithelium: Perturbation of xenobiotic metabolism, inflammation, hypoxia, apoptosis, cell proliferation.</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
</tr>
</tbody>
</table>

**PMI SCIENCE**

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. Schlagy,‡ Gregory Vuillaume,‡ Florian Martin,‡ Bjorn Titz,‡ Patrice Leroy,‡ Angar Buettner,§ Ashraf Elamin,§ Alberto Orgido,§ Maciej Cabanski,§–² Héctor De Leon,† Emmanuel Guedj,† Thomas Schneider,† Marja Talikka,† Nikolai V. Ivanov,† Patrick Vanscheeuwijk,‡ Manuel C. Peitsch,† and Julia Hoeng,†,²

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

Bjorn Titz,† ‡ 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 Ettrons,§ Reijo Laaksonen,§ Patrick Vanscheeuwijk,‡ Manuel C. Peitsch,† and Julia Hoeng,†,²

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

Justyna Szostak †, ‡ Stéphanie Boué †, Marja Talikka ‡, Emmanuel Guedj ‡, Florian Martin ‡, Blaine Phillips †, Nikolai V. Ivanov †, Manuel C. Peitsch †, Julia Hoeng

* Philip Morris International R&D, Philip Morris Products S.A., Quai JeanJacques 5, 2000 Neuchâtel, Switzerland
† Philip Morris International R&D, Philip Morris Products S.A., Quai JeanJacques 5, 2000 Neuchâtel, Switzerland
‡ Philip Morris International R&D, Philip Morris Products S.A., Quai JeanJacques 5, 2000 Neuchâtel, Switzerland
§ Philip Morris International Research Laboratories Pte Ltd, Science Park Rd, Singapore
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.
Average reductions in formation of HPHCs for CHTP 1.2 compared with levels measured in smoke from the 3R4F reference cigarette*

<table>
<thead>
<tr>
<th>Percentage Reduction (%)</th>
<th>Reference Cigarette</th>
<th>CHTP 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>WHO_IARC</td>
<td>96%</td>
</tr>
<tr>
<td>10</td>
<td>FDA</td>
<td>95%</td>
</tr>
<tr>
<td>20</td>
<td>HealthCanada</td>
<td>95%</td>
</tr>
<tr>
<td>30</td>
<td>PMI</td>
<td>94%</td>
</tr>
<tr>
<td>40</td>
<td>Carcinogens_IARC</td>
<td>96%</td>
</tr>
</tbody>
</table>

* 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.

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.

Bodyweight Curves

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

In Urine
Nicotine metabolites in exposed groups
### Disease Endpoint - Aortic Plaque Progression in ApoE−/− Mice Study 2

#### Aortic arch plaque area measurements

<table>
<thead>
<tr>
<th>Group</th>
<th>Plaque Area</th>
<th>Percentage of Plaque (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>17.9%</td>
<td>20.4%</td>
</tr>
<tr>
<td>3R4F</td>
<td>24.6%</td>
<td>18.0%</td>
</tr>
<tr>
<td>CHTP1.2</td>
<td>17.1%</td>
<td>20.4%</td>
</tr>
<tr>
<td>THS2.2</td>
<td>16.5%</td>
<td>18.0%</td>
</tr>
<tr>
<td>Cessation</td>
<td>18.0%</td>
<td>20.4%</td>
</tr>
<tr>
<td>Switch CHTP</td>
<td>17.1%</td>
<td>20.4%</td>
</tr>
</tbody>
</table>

#### Percentage of plaque (%)

- **Sham**: 20.4%<br>- **3R4F**: 18.0%<br>- **CHTP1.2**: 20.4%<br>- **THS2.2**: 18.0%<br>- **Cessation**: 20.4%<br>- **Switch CHTP**: 17.1%

![Planimetry pictures representing plaque area in aortic arch](image)

+ $p < 0.05$ significant versus sham
# $p < 0.05$ significant versus 3R4F
Inflammatory cells in BALF

Differential counts

- **Alveolar macrophages (x10^5)**
- **Alveolar dendritic cells (x10^5)**
- **Neutrophils (x10^5)**
- **Lymphocytes (x10^5)**

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

Free Lung Cells in BALF in ApoE⁻/⁻ Mice Study 2
Heart (left ventricle) Transcriptomics in ApoE^{-/-} mice study 2

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

RNA extraction

cDNA transformation+ labelling

Hybridized

Scan

Heart ventricle

3R4F

CHTP1.2

THS2.2

CESS

SWITCH

Z-score

3m 4m 6m

Volcano plot
Are the results for THS 2.2 reproducible in two independent studies?
Biomarkers of Exposure Comparison for 3R4F and THS 2.2 Across Two ApoE−/− Studies

Biomarkers of exposure in ApoE−/− Study 1 versus Study 2 for 3R4F and THS 2.2

In Urine

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F</td>
<td>REL 3R4F</td>
<td>REL THS2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>REL THS2.2</td>
<td>#</td>
</tr>
<tr>
<td>CEMA</td>
<td>REL 3R4F</td>
<td>REL THS2.2</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*#</td>
<td></td>
</tr>
<tr>
<td>SPMA</td>
<td>REL 3R4F</td>
<td>REL THS2.2</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*#</td>
<td></td>
</tr>
<tr>
<td>Total NNL</td>
<td>REL 3R4F</td>
<td>REL THS2.2</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*#</td>
<td></td>
</tr>
</tbody>
</table>

In Blood

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

* p < 0.05 significant versus sham
# p < 0.05 significant versus 3R4F
Free Lung Cells in BALF Comparison for 3R4F and THS 2.2 Across Two ApoE⁻/⁻ Studies

**Inflammatory cells in ApoE⁻/⁻ Study 1 versus Study 2 for 3R4F and THS 2.2**

**Total Cells Count**

<table>
<thead>
<tr>
<th>Study</th>
<th>6M Relative to sham</th>
<th>Mean Relative to sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Phillips et al, 2016)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2018)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Differential counts**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendritic cells</td>
<td>REL 3R4F</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>REL THS2.2</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>REL 3R4F</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>REL THS2.2</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>Macrophages</td>
<td>REL 3R4F</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>REL THS2.2</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>REL 3R4F</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>REL THS2.2</td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

* 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

* $p < 0.05$ 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)

Plaque develops in wall of artery
Plaque builds up
Plaque ruptures

<table>
<thead>
<tr>
<th>Endpoint Name (P2)</th>
<th>Group</th>
<th>6M</th>
<th>9M</th>
</tr>
</thead>
<tbody>
<tr>
<td>REL 3R4F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REL THS 2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Study 1 (Phillips et al, 2016)
Study 2 (2018)

- 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 (Phillips et al, 2015)  
Study 2 (2018)

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 (Phillips et al, 2015)  
Study 2 (2018)

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

Lung  
RNA extraction  
cDNA transformation+ labelling  
Hybridized  
Scan
Comparison for 3R4F impact and THS 2.2 across two ApoE\(^{-/-}\) studies

**Atherosclerotic plaque progression**

<table>
<thead>
<tr>
<th></th>
<th>Study 1 (Phillips et al., 2016)</th>
<th>Study 2 (2015)</th>
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<tbody>
<tr>
<td>Aortic arch - Area</td>
<td>RsL, MqL, ThL</td>
<td>ThL</td>
</tr>
<tr>
<td>Micro CT measurements</td>
<td></td>
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</tbody>
</table>

**Inflammatory cells in BALF**

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
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</thead>
<tbody>
<tr>
<td>Genotoxic cells</td>
<td>RsL, MqL, ThL</td>
<td>ThL</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>RsL, MqL, ThL</td>
<td>ThL</td>
</tr>
<tr>
<td>Macrophages</td>
<td>RsL, MqL, ThL</td>
<td>ThL</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>RsL, MqL, ThL</td>
<td>ThL</td>
</tr>
</tbody>
</table>

**Inflammatory cells in lung**

*\(p < 0.05\) significant versus sham

**Histopathology**

**Morphological changes in nose**

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**


Toxicological Sciences, 149: 411-432 (2016)
INTERVALS - enabling science to support designing a smoke-free future

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

Website http://intervals.science/
Faceted search enables quick retrieval of resource of interest

- Detailed protocols
- Study data sets
- Community features (news/commenting/events)
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 3RF-exposed mice. All 3 parameters (plaque volume, plaque area, and aortic occlusion) were significantly higher compared with sham-exposed mice, but the THS2.2 reassembled and switching groups were not different from sham (see Figures 2 and 3 below). The aorta plaque surface area (the micro-CT parameter most closely resembling the morphometric plaque area) was 78% higher for the 3RF group versus sham, while manual quantification of plaque area in the isolated aortas showed a 93% higher value following 3RF-CS exposure.

**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 area.

**Data Download:**

- 588.9 kB
- 26.8 MB

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.
The Neuchâtel Team

Collaborators

The Singapore Team