An Assessment Strategy for Modified-Risk Tobacco Products (MRTP)

February 3rd, 2017

Presented by Carine Poussin, PhD

This research was funded by Philip Morris International
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 non-combustible products ultimately replace cigarettes to the benefit of adult smokers, society, our company and our shareholders.


Figure adapted from Clive Bates presentation to E-Cigarette Summit (19 Nov 2013)

Note: Reduced-Risk Products ("RRPs") is the term we use to refer to products that have the potential to reduce individual risk and population harm in comparison to smoking combustible cigarettes.
Introduction to PMI R&D

Extensive and rigorous scientific assessment studies using quality and regulatory standards
Eliminating combustion is key...

- More than 6,000 constituents identified in cigarette smoke
- About 100 of these constituents are categorized as **harmful or potentially harmful constituents** ("HPHCs")
- Most of the HPHCs are formed when the tobacco burns
Electrically Tobacco Heating System (THS) 2.2 – Operating Principles

Heating engine precisely controlled using built-in software

- Heater maintains tobacco temperature in the distillation range (< 350 °C)
- Heater also acts as a temperature sensor

Aerosol Chemistry

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

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

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Product Design and Control Principles</td>
</tr>
<tr>
<td>2</td>
<td>Aerosol Chemistry and Physics</td>
</tr>
<tr>
<td>3</td>
<td>Standard Toxicology Assessment</td>
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<td>Systems Toxicology Assessment</td>
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<td>5</td>
<td>Clinical Trials</td>
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<td>6</td>
<td>Consumer Perception and Behavior Assessment</td>
</tr>
<tr>
<td>7</td>
<td>Post-Market Studies and Surveillance</td>
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</tbody>
</table>

- Reduced Population Harm
- Reduced Exposure & Risk
- Reduced Risk in Laboratory Models
- Reduced Toxicity in Laboratory Models
- Reduced Formation of HPHCs
Risk Framework for MRTP Assessment

- Compare **switching to a candidate MRTP** with continued smoking and benchmark against **smoking cessation** (= “gold standard” as defined by US Institute of Medicine state)

- Assess **how close switching to candidate MRTP** is to smoking cessation

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

**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**

Developing Scientific Evidence: MRTP Assessment Program

- Post-Market Studies and Surveillance
- Consumer Perception and Behavior Assessment
- Clinical Trials
- Systems Toxicology Assessment
- Standard Toxicology Assessment
- Aerosol Chemistry and Physics
- Product Design and Control Principles

Reduced Population Harm
Reduced Exposure & Risk
Reduced Risk in Laboratory Models
Reduced Toxicity in Laboratory Models
Reduced Formation of HPHCs
Systems Toxicology

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

Computational models

Apical measurements

Molecular measurements

Enabling technologies

Detailed mechanistic understanding of toxicology

Prediction of adverse outcomes

New paradigm for risk assessment

Environmental protection

Safe drugs

Green chemistry

Safe food

System Toxicology Research
Identify and Represent Disease Mechanisms

Understanding of disease onset and progression

Chronic Cigarette Smoke Exposure → Molecular changes → Disruption of Biological Mechanism → Cell / Tissue Changes → Physiological changes → Disease (CVD, COPD, Lung cancer)

Analytical Chemistry → Biological Networks – Systems Biology/Toxicology → Medicine

Literature-derived knowledge

Data-derived knowledge

Identify & Build Biological Network models

Methods - Systems Toxicology Assessment
Use Disease Mechanism Understanding for Product Assessment

Product Items and Mode of Exposure for Comparative Assessment

Conventional cigarettes:
University of Kentucky Standard Reference Cigarette 3R4F
Generation of smoke using a standard smoking protocol: Health Canada Intense

Potentially modified-risk tobacco product:
Heatsticks and Tobacco Heating System, THS 2.2

Mode of Exposure
• **Smoke / Aerosol** (*in vivo / in vitro*)
• **Smoke/ Aerosol Fractions** (*in vitro*)
  o Gas vapor phase (GVP)
  o Total particulate matter (TPM)
  o Smoke/Aerosol bubbled in aqueous solution (Aqueous extract)
Choice of *In vitro* Systems

Translation between species and experimental systems

- Relevant for investigating cellular mechanisms of diseases
- Reliable for translational biology and toxicology
- Supports the principles of 3Rs (Replacing, Reducing, and Refine)
  - 2009 European Commission a report: Alternative Testing strategies for «Replacing, Reducing, and Refining» («3R») the use of animals in research (i.e. 3D cultures, organ-on-chip)
Organotypic Bronchial, Nasal, and Oral Tissue Cultures Resemble in vivo Respiratory Epithelium


### A Series of Studies Investigating Cigarette Smoke (CS) Exposure using 3D-Organotypic Upper and Lower Airway Epithelial Tissue Cultures

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

CSE: cigarette smoke extract. Studies conducted at PMI.
## THS2.2 Aerosol Exposure Assessment using 3D Organotypic Cultures

<table>
<thead>
<tr>
<th>Author/ Year</th>
<th>Mode of Exposure</th>
<th>Duration</th>
<th>Cytotoxicity</th>
<th>Gene Expression</th>
<th>Cilia Frequency/ Function</th>
<th>Inflammatory Mediators</th>
<th>Epithelial Barrier Integrity</th>
<th>Additional endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zanetti 2016 (Buccal)</td>
<td>Single exposure of whole CS and MRTP</td>
<td>28 min</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
<td>Histology, CYP activity, miRNA profile</td>
</tr>
<tr>
<td>Iskandar 2016 (Nasal)</td>
<td>Single exposure of whole CS and MRTP</td>
<td>28 min</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Histology, CYP activity, miRNA profile</td>
</tr>
<tr>
<td>Iskandar 2017 (Bronchial)</td>
<td>Single exposure of whole CS and MRTP</td>
<td>28 min</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Histology, CYP activity, miRNA profile</td>
</tr>
</tbody>
</table>

**Conclusions:**
THS2.2 aerosol exposure has a reduced biological impact in human bronchial, nasal and oral cultures compared with 3R4F (reference cigarette) smoke at comparable nicotine concentrations
Atherosclerosis – Vascular Disorder

Development of atherosclerotic plaques over time

- Endothelium activation and dysfunction
- Leukocyte adhesion and transmigration
- Foam cell formation
- Smooth muscle cell proliferation and migration
- Coagulation
- Plaque instability and rupture
- Infarctus
- Stroke

Systems toxicology-based assessment of the candidate modified risk tobacco product THS2.2 for the adhesion of monocytic cells to human coronary arterial endothelial cells

Carine Poussin*, Alexandra Laurent, Manuel C. Peitsch, Julia Hoeng, Hector De Leon

Philip Morris International R&D, Philip Morris Products S.A., Quai Jeamnacq 5, 2000 Neuchâtel, Switzerland
In Vitro Leukocyte-Endothelial Cell Adhesion Assay - Principles

- **Mono Mac-6 cells (MM6)**

- **Human coronary artery endothelial cells (HCAECs)**
  - Disease-relevant human primary cells

### Treatment

1. **Draq5-nuclear stained MM6 cells**
2. Incubate MM6 and HCAECs together for 45 min
3. **Hoechst-nuclear stained HCAECs**
4. 15 min Wash and Fix
5. Wash and Read

Adhesion Rate measurement using Cellomics Arrayscan to count: The number of adherent MM6 cells and the number of HCAECs
Concentrations of Carbonyls are reduced in THS2.2 Aqueous Extract Compared with 3R4F While Nicotine Concentrations remain Similar

Mode of Exposure: Aqueous extracts

<table>
<thead>
<tr>
<th></th>
<th>3R4F-sbPBS</th>
<th>THS2.2-abPBS</th>
<th>3R4F/THS2.2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit: ug/item</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>42.0</td>
<td>2.8</td>
<td>15.2</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>1168.4</td>
<td>161.9</td>
<td>7.1</td>
</tr>
<tr>
<td>Acetone</td>
<td>526.2</td>
<td>29.4</td>
<td>17.8</td>
</tr>
<tr>
<td>Acrolein</td>
<td>128.3</td>
<td>7.0</td>
<td>18.3</td>
</tr>
<tr>
<td>Propionaldehyde</td>
<td>59.1</td>
<td>8.6</td>
<td>6.9</td>
</tr>
<tr>
<td>Crotonaldehyde</td>
<td>57.8</td>
<td>2.7</td>
<td>21.4</td>
</tr>
<tr>
<td>Methyl-ethyl-ketone</td>
<td>127.6</td>
<td>5.8</td>
<td>21.9</td>
</tr>
<tr>
<td>Butyraldehyde</td>
<td>21.2</td>
<td>8.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Nicotine</td>
<td>77.6</td>
<td>68.4</td>
<td>4.9</td>
</tr>
</tbody>
</table>

**Analytes highlighted in grey showed significant differences (t-test; \( p < 0.05 \)) between 3R4F and THS2.2.**

Number of independent aqueous extracts (N):
For carbonyl analysis, N=34 and 16 for 3R4F and THS2.2, respectively.
For nicotine, N=5 for both.

Poussin et al, Toxicology 339:73-86 (2016)

s/abPBS: smoke/aerosol-bubbled phosphate buffered saline
**Study Design**

- **INDIRECT**
  - THS2.2 or 3R4F Aqueous Extract
  - Treat MM6 cells (2h)
  - Collect and freeze conditioned-media
  - Trea\tHCAECs (4h)
  - Adhesion assay (functional endpoint)
  - Transcriptomits (molecular endpoints)

- **DIRECT**
  - MM6 starvation medium (2h)
  - Unconditioned-media
  - HCAECs

- **FRESH DIRECT**
  - THS2.2 or 3R4F AE
  - Monocyte-derived molecules
  - Stable aerosol chemicals
  - Unstable aerosol chemicals

Poussin et al, Toxicology 339:73-86 (2016)
The Release of Inflammatory Mediators by Monocytic Cells is Reduced with THS2.2 Compared with 3R4F

TNF-alpha measured in MM6 conditioned-media

**p≤0.05,** p≤0.01, ***p≤0.0001 vs. 0 puffs/ml (vehicle control)

Mean ± SEM

Poussin et al, Toxicology 339:73-86 (2016)
Reduced Effects of THS2.2 Compared with 3R4F on the Adhesion of Monocytic Cells to Coronary Artery Endothelial cells

**p≤0.05, ***p≤0.0001 vs. 0 puffs/ml (vehicle control)
Reduced Biological Impact of THS2.2 Compared with 3R4F on Human Coronary Artery Endothelial Cells

Poussin et al, Toxicology 339:73-86 (2016)
Underlying Mechanisms

**How many adherent monocytes?**

- **3R4F**
  - Low
  - $\leq 20$

- **THS2.2**
  - Low/High
  - $\leq 20$

**What mechanism(s)?**

- **Inflammatory-driven adhesion**
  - Endothelial cells

- **Cell Stress (cytotoxicity)-driven adhesion**
Case Study 2: Assessment of THS2.2 compared with 3R4F on the Migratory Behavior of Monocytic cells (THP-1)

Food and Chemical Toxicology 85 (2015) 81–87

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short communication

Aerosol from a candidate modified risk tobacco product has reduced effects on chemotaxis and transendothelial migration compared to combustion of conventional cigarettes

Marco van der Toorn, Stefan Frentzel, Hector De Leon, Didier Goedertier, Manuel C. Peitsch, Julia Hoeng

Philip Morris International S.A., Philip Morris Products S.A., Quai Jean-Jacques S. 2000 Neuchatel, Switzerland
Reduced Effects of THS2.2 Compared with 3R4F on Monocytic Cell Cytotoxicity and Inflammatory Mediators Production

THP1 cells

Cytotoxicity

Inflammatory mediators

Van der Toorn et al, Food and Chemical Toxicology 86:81-87 (2015)
Reduced Effects of THS2.2 Compared with 3R4F on Transmigration of Monocytic Cells

Transwell chemotaxis (Cell counting)

A/B
Seeded THP1 cells
Migrated THP1 cells remain in suspension.

C/D
Seeded THP1 cells
HCAEC monolayer
Migrated THP1 cells adhere underneath the electrodes
Microelectrodes under porous membrane

Van der Toorn et al, Food and Chemical Toxicology 86:81-87 (2015)
Conclusions – *In vitro* Adhesion and Transmigration Studies

- Reduced effects *in vitro* of THS2.2 compared with 3R4F (at matching nicotine concentrations) on **cellular mechanisms relevant for the development of atherosclerosis**

- The concentrations of THS2.2 had to be increased by 10 to 20 times depending on the context to observe similar effects as those induced by 3R4F
Rodent Models for Assessment

- Respond to cigarette mainstream smoke
- Develop pathologies with characteristics of smoking-induced human diseases such as:
  - *Lung inflammation*, altered pulmonary function, emphysema in Chronic obstructive pulmonary disease (COPD) → C57Bl6 mouse strain
  - *Atherosclerosis* in cardiovascular diseases (CVD) → Apoe⁴⁻ mouse strain (C57Bl6 background)
  - Tumor development in Lung Cancer → A/J mouse strain


STUDY OBJECTIVES

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

STUDY DESIGN: 5 exposure groups

MEASUREMENTS

- In-life observations, body weight
- Markers of exposure: in plasma (e.g. carboxyhemoglobin, nicotine and cotinine) and urine e.g. (nicotine metabolites)
- Hematology
- Clinical chemistry (e.g. cholesterol, HDL, LDL, Glucose)
- Respiratory endpoints (BALF cell count, lung histopathology, function, volume)
- Cardiovascular endpoints (Aortic plaque volume, area, occlusion)
- Molecular analysis: Omics (Transcriptomics, Proteomics, Lipidomics) (various tissues and biofluids)
Case Study 3: ApoE⁻/⁻ Mouse Inhalation and Switching Study

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,† Bjorn Titz,† Patrice Leroy,† Ansgar Buettner,§ Ashraf Elamin,† Alberto Oviedo,* Maciej Cabanski,†,† Héctor De León,† Emmanuel Guedj,† Thomas Schneider,† Marja Talikka,† Nikolai V. Ivanov,† Patrick Vanscheeuwijk,† Manuel C. Peitsch,† and Julia Hoeng,†,‡
Reduced Effects of THS2.2 Compared with 3R4F on the Growth of Aortic Arch Plaques

Aortic arches were dissected, longitudinally opened, pinned down, and stained with OilRedO for planimetry.

# Systems Toxicology Mouse Inhalation Studies (IS) for MRTP Assessment

*MRTP = modified-risk tobacco product*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Study</th>
<th>Reference</th>
<th>Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>7-month IS: MRTP = SMAR (prototype)</td>
<td>Philips et al, 2015, PMID 25843363</td>
<td>Study description / Respiratory disorders</td>
</tr>
<tr>
<td></td>
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<td>Ansari et al, 2016, PMID 26731301</td>
<td>Data description and availability</td>
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<td>Elamin et al, 2016, PMID 27268958</td>
<td>Lung proteome</td>
</tr>
<tr>
<td>Apoe-/-</td>
<td>8-month IS: MRTP = THS2.2</td>
<td>Phillips et al, 2016, PMID 26609137</td>
<td>Cardiovascular and respiratory disorders</td>
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<td>Szostak et al, 2017, PMID 28111298</td>
<td>Heart transcriptomics</td>
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<tr>
<td></td>
<td></td>
<td>Lasso et al, 2016, PMID 27027324</td>
<td>Liver transcriptomics</td>
</tr>
<tr>
<td>Both models</td>
<td>Mentioned above</td>
<td>Titz et al, 2016, PMID 26582801</td>
<td>Integrative systems toxicology: lung lipidomics, proteomics and transcriptomics</td>
</tr>
</tbody>
</table>
Systems Toxicology Animal Inhalation Studies (IS) for MRTP Assessment

- Rat inhalation studies according to regulatory guidelines (*TG 413) from the Organization for Economic Cooperation and Development (OECD) – Executed according to GLP
  - **Goal**: to assess the degree of reduced exposure in laboratory models
  - **Measurements**: Standard and systems toxicology endpoints
  - The results show reduced exposure and effects of THS2.2 compared with 3R4F in laboratory models

- Publications:
  - Sewer et al, Regul Toxicol Pharmacol, 81(S2): S82-S92 (2016)
Systems Toxicology - Conclusions

This research was funded by Philip Morris International
In comparison with the 3R4F reference cigarette, the data shows that:

- THS2.2 yield significantly lower levels of harmful and potentially harmful constituents (HPHCs) on average of about 90-95%

- This leads, in laboratory models (*in vitro* and *in vivo*), to:
  - Reduced exposure to HPHCs and reduced toxicity in laboratory models
  - Reduced biological impact on key cellular mechanisms (i.e. endothelial inflammation)
  - Reduced impact on disease-associated mechanisms (reduced severity of disease endpoints) (i.e. aortic plaque growth)

- The effects of Switching from 3R4F to THS2.2 approach those observed in Cessation in these models
The Relationship between PMI’s Non-Clinical and Clinical MRTP Assessment Programs

This research was funded by Philip Morris International
The Relationship between PMI’s Non-Clinical and Clinical MRTP Assessment Programs

• **Developed** using the body of scientific literature on:
  – Epidemiological evidence linking smoking and disease
  – Mechanistic understanding of disease onset and progression associated with smoking

• **Aligned**, so that evidence generated in:
  
  | Non-clinical: biomarkers of exposure + mechanistic endpoints |
  |
  | Corroborated | Expanded |
  |
  | Clinical: biomarkers of exposure + clinical risk endpoints |
Case Study 4: Reduced Exposure Clinical Study in Japan

**Adult smokers used the products ad libitum**

Adult smokers randomized to cigarettes or THS2.2 were free to use the product as often as they wished, in confinement (5 days) and then ambulatory (85 days).

Levels of reduced exposure approached those observed in people who stopped smoking for the duration of the studies.

Note: These data alone do not represent a claim of reduced risk
Source: PMI Research and Development
Registered on clinicaltrials.gov: NCT01970995
Transparency and Independent Verification

This research was funded by Philip Morris International
## Transparency and Independent Verification of PMI’s Results

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Independent Studies</strong></td>
<td>Launched an investigator-initiated studies program that supports <strong>external scientists</strong> who can conduct independent research related to our MRTPs through the provision of products, equipment, and financial or technical support.</td>
</tr>
<tr>
<td><strong>Independent Verification</strong></td>
<td>Crowd-sourced verification of data and methods using double blind assessment <a href="http://sbvimprover.com">sbvimprover.com</a></td>
</tr>
<tr>
<td><strong>Independent Expert Review</strong></td>
<td>Conducting in-depth analysis of study reports by independent experts</td>
</tr>
<tr>
<td><strong>Publish Results</strong></td>
<td>&gt; 200 peer-reviewed articles since 2008 <a href="http://pmiscience.com">pmiscience.com</a></td>
</tr>
</tbody>
</table>
sbv IMPROVER

Project initiated 6 years ago and funded by Philip Morris International

Aims to provide a measure of quality control in R&D by identifying the building blocks that need verification in a complex industrial research pipeline

Aims to verify methods & data in systems biology / toxicology using double blind performance assessment

Complements the classical peer review system

Verification of systems biology research in the age of collaborative competition

Pablo Meyer¹, Leonidas G Mparmpopoulos², Thomas Bonk¹, Andrea Califano³, Carolyn R Cho⁴, Alberto de la Fuente¹, Daniel de Graaf⁵, Alexander J Horvath⁶, Julia Hoeng⁷, Nikola V Karamouz⁸, Heliz Kocatepe⁹, Duan Linding⁷, David Marbach⁵, Raquel Nodel⁴, Manuel C Peitso¹, I Jeremy Rice⁴, Ajay Royyuru¹, Frank Schachner¹,², Joerg Sprengel⁵, Katrin Stiller⁴, Dennis Vilkup⁴, and Gustavo Stolovitzky²

Collaborative competitions, in which communities of researchers compete to solve challenges may facilitate more rigorous scrutiny of scientific results.

Nature Biotechnology 2011 Sep;29(9):811-5

sbvimprover.com
sbv IMPROVER leverages the crowd to complement the classical peer review system
Past sbv IMPROVER computational challenges

**Diagnostic signature challenge (2012)**
To identify gene signatures for diagnostic classification in four disease area

PUBLICATIONS
- Tarca et al, Bioinformatics 29 (22) (2013)
- Special issue in Systems Biomedicine 1 (4) (2013) including 11 articles

**Species translation challenge (2013)**
To identify and quantify a function of translatability of biological perturbations across human and rodent species

PUBLICATIONS
- Special Issue in Bioinformatics 31 (4) (2014) including 6 articles
- Poussin et al, Scientific Data 1:140009 (2014)

**Network verification challenge (2014-2015)**
To review biological network models that are suitable for drug discovery, toxicological and mechanistic research in respiratory disease

PUBLICATIONS
- sbv IMPROVER projet team, Gene Regul Syst Bio (2016)
- sbv IMPROVER project team, Pac Symp Biocomput (2015)

**Systems Toxicology challenge (2015-2016)**
To identify robust blood-based gene signatures as predictors for smoking and cessation status

PUBLICATIONS
- 3 other manuscripts in preparation

sbvimprover.com
Case Study 5: Whole Blood Exposure Response Marker Identification: Crowdsourcing validation of PMI’s gene expression signature

sbv IMPROVER Systems Toxicology Computational Challenge\(^{(1)}\)

\(^{(1)}\) Poussin et al. Chemical research in toxicology. 2017
\(^{(2)}\) Martin et al. Human & Experimental Toxicology. 2015

- 135 registered participants
- 61 international teams
- 23 valid submissions

**similar results were obtained for REX-C-04-JP (not shown)**
**selected by at least 2 teams**
**significantly different from smoke exposed group**

Gene Signature

\[ LRRN3, SASH1, PALLD, RGL1, \]
\[ TNFRSF17, CDKN1C, IGJ, RRM2, ID3, \]
\[ SERPING1, FUCA1 \]

In black genes common to the crowdsourcing signature**

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S / 3R4F: Smoker / 3R4F exposed
FS / Cess: Former Smoker / Cessation
NS / Sham: Never Smoker / exposed to fresh air
SA: Smoking Abstinence
THS2.2: THS2.2
Switch: Switch to THS2.2
Summary and Conclusions

This research was funded by Philip Morris International
Conclusions

Our scientific assessment program enabled us to determine already that **THS2.2**: 

- **Does not generate combustion** through normal operation
- Generates an aerosol with, **on average, 90-95% lower levels of HPHCs** compared with reference cigarette smoke
- **Is on average 90-95% less toxic** in laboratory-based tests compared with reference cigarette smoke
- **Reduces the risk of smoking-related diseases** in sophisticated laboratory-based models
- Adults smokers **switching to THS2.2**:  
  - have **reduced biomarkers of exposure (HPHCs)** compared with adult smokers who continued smoking  
  - their **levels approached those observed in smokers who quit smoking** for the duration of the study

**December 6th, 2016: submission of a MRTP application for THS2.2** with the U.S. Food and Drug Administration’s Center for Tobacco Products
Thank you for your attention

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