Cardiovascular Effects Observed When Using the Tobacco Heating System (THS) 2.2 Compared with Continued Smoking

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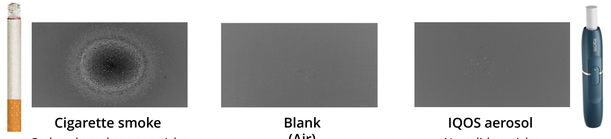
Introduction and Objectives

Background

Cigarette smoking is causally linked to the development of cardiovascular disease (CVD). Cigarette smoke (CS) contains 93 chemicals categorized as harmful and potentially harmful constituents (HPHC) by the U.S. Food and Drug Administration. Twelve HPHCs have been identified as cardiotoxic. The HPHCs, oxidants, and carbon-based nanoparticles (cbNPs) contained in CS are mediators of endothelial dysfunction, vascular inflammation, oxidative stress, and other pathological mechanisms underlying atherosclerosis and CVD. To reduce the risk of CVD and other smoking-related diseases, Philip Morris International has developed the Tobacco Heating System (THS) 2.2, which heats a tobacco plug in a controlled manner, never allowing the temperature to exceed 350°C, preventing the combustion process from taking place, thereby generating an aerosol containing no cbNPs and significantly reduced levels of HPHCs (cardiovascular toxicants are reduced by > 92% in THS 2.2 aerosol) vs. CS.



Cigarette smoke
Carbon-based nanoparticles
Median diameter = 75 nm
Amount: $6x10^{11}$ particles ~= 0.7 mg*

Blank (Air)

IQOS aerosol
No solid particles

* Under the Health Canada Intense smoking regime.

Our assessment program aims to demonstrate that switching to THS 2.2 has the potential to reduce the risk of smoking-related diseases vs. continued smoking. This-program includes toxicology tests that follow Organisation for Economic Co-operation and Development guidelines, Good Laboratory Practice, a systems toxicology approach, and randomized, controlled clinical studies following Good Clinical Practice.

Methods

Clinical Study — Clinical Risk Endpoints in THS Switchers[1]

A randomized, controlled, two-arm parallel group, multicenter, U.S. study was conducted over six months in adult smokers who switched from cigarettes to THS 2.2, compared with those who continued to smoke CCs, to demonstrate favorable changes in THS 2.2 users (≥ 70%) in eight co-primary endpoints representative of pathomechanistic pathways (e.g., of inflammation, lipid metabolism, endothelial function, platelet function, and oxidative stress involved in CVD). 984 subjects were randomized to CC (n = 496) or THS 2.2 (n = 488).

Adhesion of Monocytes to Human Coronary Arterial Endothelial Cells (HCAEC), a Critical Stage in Atherosclerosis — THS 2.2 vs CS (*In Vitro* Adhesion Assay)[2]

Cell exposure to 3R4F reference cigarette or THS 2.2 aqueous smoke/aerosol extract (smoke-/aerosol-bubbled phosphate-buffered saline [PBS] [sbPBS/abPBS])

Conditioned and unconditioned media preparation

Monocytic (MM6) cells were starved in medium (two hours), then exposed to 3R4F reference cigarette or THS 2.2 sbPBS (or PBS) for two hours. Both media were frozen.

Treatment of HCAECs

Indirect and direct treatments: 24-hour-starved HCAECs were treated with thawed conditioned and unconditioned media for four hours. Fresh direct treatment: 24-hour-starved HCAECs were exposed to freshly generated 3R4F or THS 2.2 sbPBS (or PBS) for four hours. HCAECs and MM6 lysates were collected and stored at –80°C for RNA extraction.

Adhesion Assay

Untreated MM6 cells and four-hour-treated HCAECs were nuclear stained for 15 minutes and then incubated together for 45 minutes. After cell fixing and washing, remaining adherent MM6 and HCAEC cells were counted, and adhesion rate was calculated.

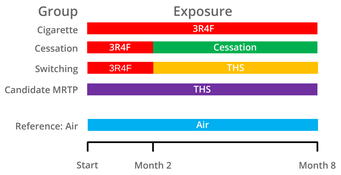
Methods

In Vivo Study to Investigate Atherosclerotic Plaque of the Aortic Arch[3]

This study examined the development of the hallmarks of CVD in ApoE$^{-/-}$ mice chronically exposed to 3R4F, THS 2.2 aerosol (matched to the nicotine concentration in 3R4F [30 µg/l]), or filtered air for three hours per day, five days per week, for up to eight months (approximately 40% of lifetime).

After two months of exposure to 3R4F, mice were switched to THS 2.2 aerosol (switching), filtered air (cessation), or continued exposure to 3R4F.

The exposure dose corresponded to ~30 cigarettes per day in human comparison.

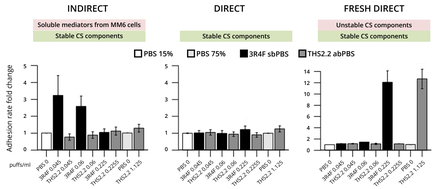


Results

Clinical Study: Changes in Clinical Risk Endpoints

Endpoint	Change from CC Use	Expected Change (used for sample size calculation)	Observed Change LS Mean Difference/Relative Reduction	Hailperin-Rüger Adjusted 96.875% CI	1-Sided *p*-value (0.0156)	THS 2.2 Directional Change vs. SA (literature)
HDL-C	Difference	3.3 mg/dL	3.09 mg/dL	1.10, 5.09	< 0.001*	✓ significant
WBC Count	Difference	–0.6 GI/L	–0.420 GI/L	–0.717, –0.123	0.001*	✓ significant
sICAM-1	% Reduction	12%	2.86%	–0.426, 6.04	0.030	✓
11-DTX-B2	% Reduction	18%	4.74%	–7.50, 15.6	0.193	✓
8-epi-PGF$_{2\alpha}$	% Reduction	16%	6.80%	–0.216, 13.3	0.018	✓
COHb	% Reduction	65%	32.2%	24.5, 39.0	< 0.001*	✓ significant

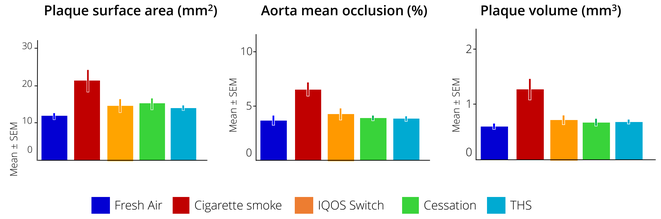
In Vitro Model: Adhesion Assay

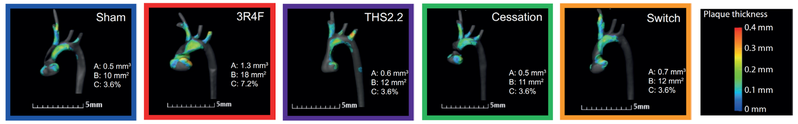


- 3R4F aqueous cigarette smoke extract promoted adhesion of MM6 cells to HCAECs in indirect and fresh direct exposure conditions.
- At the same concentrations, no significant adhesion of MM6 cells to HCAECs (reduced by exposure to THS 2.2) was observed following THS 2.2 abPBS treatment.
- To observe similar effects as 3R4F, at functional and molecular levels, concentrations of THS 2.2 required a concentration increase of ~10 and 20 times.

Results

In Vivo Model: Atherosclerotic Plaque in the Aortic Arch Data from µCT at Month 7





µCT scanned aorta after the staining (3D reconstruction showing position and the thickness of the plaque).

A: Aorta plaque volume (mm³); **B**: Aorta plaque surface area (mm²); **C**: Aorta mean occlusion (%)

Conclusions

The results of the THS 2.2 translational assessment program demonstrate that:

- THS 2.2 aerosol contains no cbNPs. Additionally, cardiovascular toxicants are reduced by > 92%.
- The effects of THS 2.2 aerosol on the adhesion of monocytic cells to human coronary endothelial cells *in vitro* are significantly reduced.
- Switching to THS 2.2 halted the progression of CS-induced atherosclerotic changes. THS 2.2 aerosol had minimal adverse effects in the ApoE$^{-/-}$ mouse study.
- In humans, all co-primary endpoints linked to smoking-related disease shifted favorably, in the same direction as the smoking cessation effect observed in the literature, after six months of switching from cigarettes to THS 2.2.

PMI has completed 17 non-clinical studies and 9 clinical studies, including the studies presented here. The totality of evidence available to date, from non-clinical and clinical studies, indicates that switching to THS 2.2 has the potential to reduce the risk of smoking-related diseases, such as CVD.

References

1. Philip Morris Products S.A. "Evaluation of biological and functional changes in healthy Smokers after switching to THS 2.2 for 26 weeks [ZRHR-ERS-09-US]." In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2015- [cited 2015 Aug 26]. Available from: https://clinicaltrials.gov/ct2/show/NCT02396381?term=NCT02396381&rank=1 NLM Identifier: NCT02396381.
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3. Phillips, Blaine, et al. "An 8-month systems toxicology inhalation/cessation study in Apoe−/− mice to investigate cardiovascular and respiratory exposure effects of a candidate modified risk tobacco product, THS 2.2, compared with conventional cigarettes." Toxicological Sciences 149.2 (2015): 411-432.

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