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

C. Pater, C. Haziza, A. Elamin, M. S. Ansari, S.Pouly, G. de La Bourdonnaye, C. T. Tran, F. Lüdicke

PMI R&D, Morris Products SA, Quai Jeanrenaud 5, CH-2000, Neuchâtel, Switzerland

Introduction and Objectives

Cigarette Smoke (CS) is causally linked to the development of cardiovascular disease (CVD) through different pathophysiologic pathways, which include endothelial injury and dysfunction, oxidative stress, a procoagulatory status, inflammation, and an abnormal lipid profile, all contributing to the development of atherosclerosis.

Tobacco harm reduction, by substituting cigarettes with less harmful products, is a complementary approach to current strategies for smokers who would otherwise continue to smoke. The Tobacco Heating System (THS) 2.2 is a novel tobacco product that heats tobacco instead of burning it, never allowing the temperature to exceed 350°C, thereby preventing the combustion process from taking place and producing substantially lower levels of toxicants (on average more 90%) compared with CS. In particular, the levels of eight cardiovascular toxicants (acrolein, benz(a)anthracene, benzene, butyraldehyde, hydrogen cyanide, lead, phenol, propionaldehyde) are reduced by >92% in THS aerosol versus CS.

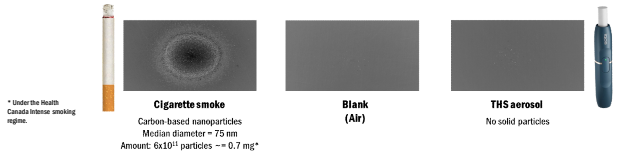


Figure 1: The THS generates an aerosol that does not contain carbon-based nanoparticles (CBNP).

Philip Morris International's (PMI) assessment program aims to demonstrate that switching to THS has the potential to reduce the risk of smoking-related diseases versus continued smoking. The program includes *in vitro*/*in vivo* toxicology testing methods that follow OECD guidelines, Good Laboratory Practice, a systems toxicology approach, and randomized, controlled clinical studies that follow the principles of Good Clinical Practice.

Methods

Adhesion of Monocytes to Human Coronary Arterial Endothelial Cells (HCAEC), a Critical Stage in Atherosclerosis — THS 2.2 vs CS (*In Vitro* Adhesion Assay)[1]

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) and 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.

In Vivo Study to Investigate Atherosclerotic Plaque of the Aortic Arch[2]

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.

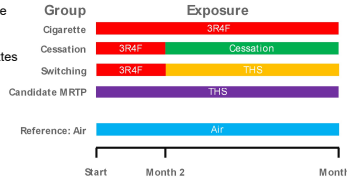


Figure 2: Groups and exposures.

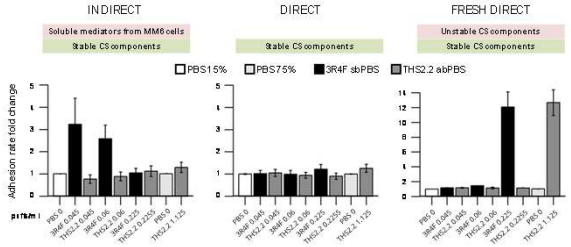
Clinical Study — Clinical Risk Endpoints in THS Switchers[3]

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 cigarettes, to demonstrate favorable changes in THS 2.2 users (≥70%) in eight co-primary endpoints representative of pathomechanistic pathways leading to atherosclerosis (e.g., inflammation, lipid metabolism, endothelial function, platelet function, and oxidative stress). 984 subjects were randomized to continued cigarette smoking (n=496) or THS 2.2 (n=488).

Results

In Vitro Model: Adhesion Assay

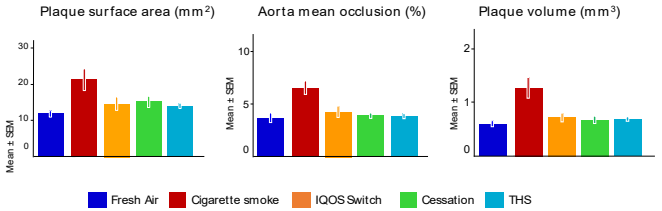
- 3R4F aqueous cigarette smoke extract promoted adhesion of MM6 cells to HCAECs in indirect and fresh direct exposure conditions.
- At the same concentrations, no significant adhesion of MM6 cells to HCAECs was observed following THS 2.2 treatment.
- To observe similar effects as 3R4F, concentrations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



*$p \leq 0.05$, ***$p \leq 0.001$ vs. 0 puffs/ml (PBS 15% or 75%).

Figure 3: Effects of THS 2.2 and 3R4F aqueous extracts on the adhesion of MM6 cells to HCAECs following indirect, direct, and fresh direct treatments of HCAECs.

In Vivo Model: Atherosclerotic Plaque in the Aortic Arch Data from Micro Computed Tomography (µCT) at Month 7



*: different from Sham ($p<0.05$), #: different from cigarette smoke ($p<0.05$)

Figure 4: Aortic arch plaque area measurements.

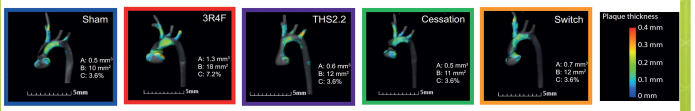


Figure 5: µCT scanned aorta after the staining (3D reconstruction showing position and thickness of the plaque).

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

Clinical Study: Changes in Clinical Risk Endpoints at Month 6

Endpoint	Change from CC Use	Expected Change (used for sample size calculation)	Observed Change LS Mean Difference/Relative Reduction	Hailperin-Ruger 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

Table 1: Changes in clinical risk endpoints at Month 6.

Conclusions and Discussion

The results of the THS 2.2 assessment program demonstrate that:

- THS 2.2 aerosol contains no CBNPs. Additionally, cardiovascular toxicants are reduced by >92%.
- The adhesion of monocytic cells to HCAECs *in vitro* is significantly reduced following THS 2.2 treatment.
- Switching to THS 2.2 halted the progression of CS-induced atherosclerotic changes *in vivo*.
- In humans, all co-primary endpoints representative of different pathophysiologic pathways leading to atherosclerosis 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 nie clinical studies, including the studies presented here.

The evidence available to date indicates that switching to THS has the potential to reduce the risk of smoking-related diseases, such as CVD.

As a next step, PMI will complement its THS assessment program with cardiovascular outcome studies intended to demonstrate the clinical benefits of switching to THS (e.g., reduction in the risk of cardiovascular death, myocardial infraction, and stroke) as compared with continued smoking and help to improve primary and secondary cardiovascular disease prevention in clinical practice.

References

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