Xenobiotic metabolism response to 3R4F or THS 2.2 exposure: from pre-clinical to clinical

S. Pouly, M. Talikka, A. van der Plas, B. Titz, A. Iskandar, G. de La Bourdonnaye, N. Blanc, F. Martin, C. Haziza, N.V. Ivanov, F. Lüdicke, J. Hoeng, M.C. Peitsch

PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

Introduction and objectives

Cytochrome P450 (CYP) enzymes are prominent xenobiotic metabolism enzymes that detoxify or activate xenobiotic compounds. The CYP1A2 enzyme is involved in the metabolism of about 9% of marketed drugs [1]. Cigarette smoking, through exposure to polycyclic amino hydrocarbons (PAH), has been shown to induce CYP1A2 enzyme activity [2], and its levels are elevated in heavy smokers compared to non-smokers [3]. Sudden smoking cessation and the subsequent abolishment of CYP1A2 induction has been documented in numerous case reports to be associated with adverse drug reactions [4].

Philip Morris International (PMI) has developed a range of Reduced-Risk Products (RRP) that present, are likely to present, or have the potential to present less risk of harm to adult smokers who switch to these products versus continuing smoking. One of these products is the Tobacco Heating System (THS 2.2, currently marketed in numerous countries under the brand name *IQOS*® with *HEETS*®), a candidate Modified Risk Tobacco Product (MRTP) for which PMI has implemented an assessment program that follows the U.S. Food and Drug Administration MRTP Applications Draft Guidance [5].

PMI assessment studies have evaluated *Cyp1a2* gene and protein expression in animal models, CYP1A1/1B1 activity in human organotypic cultures, and CYP1A2 activity in humans. Harmful and potentially harmful constituents (HPHC) of cigarette smoke (CS), including PAHs, are reduced by 90%–95%, on average, in THS 2.2 aerosol compared with CS from a standard 3R4F reference cigarette [6].

Here, we assess to which extent the lower HPHC levels in THS 2.2 aerosol, compared with CS, are accompanied by a lower engagement of xenobiotic processes, especially CYP1A2/1, upon exposure in pre-clinical and clinical studies carried out by PMI.

Methods

The pre-clinical exposure studies evaluated protein and mRNA expression changes for *Cyp1a2* in liver for two ApoE$^{-/-}$ mouse studies [7, 14]. Female ApoE$^{-/-}$ mice were exposed for up to six or eight months to fresh air (Sham), 3R4F CS, or THS 2.2 aerosol at matched nicotine concentrations.

Human organotypic nasal cultures were exposed to fresh air (Sham), 3R4F CS, or THS 2.2 aerosol at the air-liquid interface [13]. Combined CYP1A1/1B1 activity was measured using a non-lytic P450-Glo™ assay (Promega, Madison, WI, USA).

The clinical studies included two five-day [8,9] and two 90-day reduced exposure studies [10-12]:

- The five-day studies described the changes in CYP1A2 enzymatic activity on Day 5 in smokers i) switching from regular cigarettes to THS 2.2 with regular (non-menthol) heatsticks, ii) continuing to smoke regular cigarettes, and iii) smoking abstinence (SA).
- The 90-day studies described the change in CYP1A2 enzymatic activity (on Days 5 & 90) in smokers i) switching from mentholated cigarettes to THS 2.2 with menthol heatsticks (M), ii) continuing mentholated cigarette smoking, and iii) SA. The results presented refer to the per protocol (PP) populations.
- The measurement of enzyme activity was assessed through paraxanthine and caffeine (CAF) plasma molar concentrations approximately six hours (± 15 minutes) after the intake of either one cup of coffee made from 4.2 g (± 10%) regular instant coffee (Nescafé Gold Instant; Nestlé; Germany; CAF content: 72 mg/2 g) with 150 ml ±10 ml water or one Tomerumin® CAF tablet with 150 ml ± 10 ml of water.

Results

- Compared to Sham exposure, elevated levels of *Cyp1a2* were observed in the livers of ApoE$^{-/-}$ mice exposed to CS from the 3R4F reference cigarette but not in mice exposed to THS 2.2 aerosol at matching nicotine concentrations (Table 1). Upon both cessation and switching to THS 2.2, the upregulation of *Cyp1a2* observed upon CS exposure reverted to levels close to Sham.
- A causal biological network model that captures the xenobiotic network response demonstrated weaker perturbation upon THS 2.2 exposure compared with CS exposure in the lung of ApoE$^{-/-}$ mice (Figure 1).
- The combined enzymatic activity of CYP1A1/B1 was lower in human organotypic nasal cultures exposed to THS 2.2 aerosol than in those exposed to 3R4F CS (Figure 2).
- In four clinical studies conducted with THS 2.2, CYP1A2 activity was lower in the plasma of subjects who abstained from smoking or switched to THS 2.2 for five days compared with subjects who continued smoking (Table 2 and Figure 3).

Table 1. Cyp1a2 expression in ApoE$^{-/-}$ mouse liver upon CS and THS 2.2 exposure.

Cyp1a2 expression in liver	CS Group[1]	FC[2] mRNA	FC[2] protein	THS 2.2 (M) Group[1]	FC[2] mRNA	FC[2] protein	CESS/SWITCH Group[1]	FC[2] mRNA	FC[2] protein
ApoE$^{-/-}$ mouse study #1	3R4F 6m	1.39	**1.60***	THS 2.2 6m	1.05	0.96	CESS 6m	1.04	1.01
	3R4F 8m	1.44	**1.76***	THS 2.2 8m	1.11	1.00	CESS 8m	1.07	1.00
							SWITCH 6m	1.05	1.02
							SWITCH 8m	1.09	1.01
ApoE$^{-/-}$ mouse study #2	3R4F 6m	**1.35***	**1.38***	THS 2.2 6m	1.06	1.05	CESS 6m	1.02	0.97

LEGEND

*1: Group comparisons versus Sham-exposed animals labeled as item – ; 2: Expression fold changes versus Sham-exposed animals; *, False discovery rate-adjusted p-value < 0.05; Included studies: ApoE$^{-/-}$ mouse study #1 [7], ApoE$^{-/-}$ mouse study #2 [14], Group labels: Item – Time point; CESS, cessation; SWITCH, switching from 3R4F CS to THS 2.2 aerosol.*

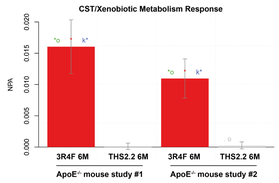

Figure 1. Xenobiotic response in lung for ApoE$^{-/-}$ mouse studies.

Network enrichment analysis for the xenobiotic metabolism response. Bars show overall network perturbation amplitude (NPA) based on transcriptomic data; error bars indicate 95% confidence intervals. Three statistical measures are shown: the red star indicates statistical significance with respect to biological replicates, the green star (o statistic) indicates significance with respect to permutation of genes downstream of network nodes, and the blue star (k statistic) indicates significance with respect to permutation of the network topology ($p < 0.05$).

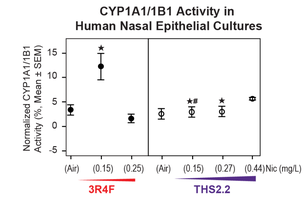

Figure 2. CYP1A1/1B1 activity in human nasal epithelial cultures.

Mean activity levels of CYP1A1/CYP1B1 (combined). The activity levels were normalized relative to the positive control (TCDD-treated cultures considered as 100% activity). Deposited nicotine concentrations in the smoke or aerosol are indicated for each group (mg/L, x-axis).

Figure adapted from [13].

Measurement was conducted 72 h following exposure
★ p-values ≤ 0.05 vs air-exposed controls
p-values ≤ 0.05 vs 3R4F (0.15)
The concentrations of nicotine (mg/L) was measured in the smoke/aerosol

Table 2. Demographic baseline characteristics of clinical study participants (PP populations).

	5-day study in Poland	5-day study in Japan	90-day study in Japan	90-day study in US
Number of participants				
THS 2.2 arm (N)	80	80	76	75
CC arm (N)	41	40	42	35
SA arm (N)	39	40	39	24
Sex				
THS 2.2 arm (%; men / women)	48.8 / 51.3	50 / 50	56.6 / 43.4	61.3 / 38.7
CC arm (%; men / women)	51.2 / 48.8	50 / 50	59.5 / 40.5	57.1 / 42.9
SA arm (%; men / women)	51.3 / 48.7	50 / 50	56.4 / 43.6	62.5 / 37.5
Mean age				
THS 2.2 arm (years; (SD))	35.4 (9.4)	37.6 (11.7)	37.2 (10.7)	39.0 (11.8)
CC arm (N) (years; (SD))	32.6 (10.1)	37.2 (11.7)	37.4 (11.2)	34.1 (10.5)
SA arm (N) (years; (SD))	33.6 (11.5)	35.9 (10.6)	37.4 (11.2)	40.5 (10.8)

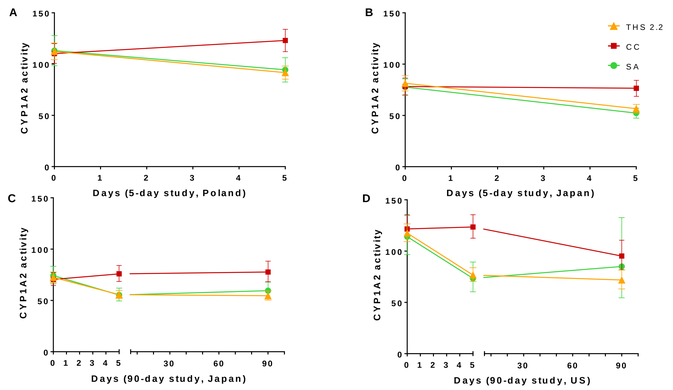

Figure 3. CYP1A2 activity in smokers, switchers to THS 2.2, and abstainers (SA) in PMI's clinical studies (PP populations).

(A) 5-day study in Poland, (B) 5-day study in Japan, (C) 90-day study in Japan, THS 2.2 Menthol, (D) 90-day study in US, THS 2.2 Menthol. A, B: arithmetic mean values and 95% confidence intervals; C, D: Geometric means (and 95% CI) were calculated based on an assumption of lognormal distribution.

Discussion

In the pre-clinical studies, exposure to THS 2.2 aerosol did not result in significant differential expression of *Cyp1a2* in the liver of ApoE$^{-/-}$ mice, engaged the xenobiotic response in the lung to a much lower extent than 3R4F CS, and was associated with lower activity of CYP1A1/B1 than 3R4F CS in human nasal organotypic cultures. The clinical studies assessing CYP1A2 enzymatic activity in smokers switching to THS 2.2 and after smoking cessation showed reductions in CYP1A2 in switchers to THS 2.2 comparable to those seen in those who quit smoking.

Smoking of 10–20 cigarettes per day appears to be a weak to moderate inducer of CYP1A2, depending on the contribution ratio values for CYP1A2 (CRCYP1A2) of the substrate drug. In smokers, drugs that are primarily metabolized by CYP1A2 will have faster systemic clearance as a result of enzyme induction [2].

On the other hand, there is substantial evidence showing that smoking cessation results in downregulation of the CYP1A2 enzyme, as it has been shown to reverse induced hepatic enzyme levels to normal [4]. After sudden smoking cessation, initial CAF clearance decreased, and the apparent half-life of CYP1A2 activity was 38.6 hours (27.4–54.4 hours). Therefore, after smoking cessation, there might be a need for dose adjustment of drugs metabolized by CYP1A2.

Data from case reports of smokers switching to e-cigarette use have shown a similar decrease in CYP1A2 activity.

There are multiple cases of elevations of CYP1A2-metabolized drug levels in those who stopped smoking, in particular in those who were taking Clozapine. The same was observed in those who switched to e-cigarettes.

Nicotine replacement therapy to assist smoking cessation will not improve this effect, because the effect on hepatic microsomal enzymes is related not to nicotine but rather to PAHs.

Even though current cessation guidelines do not mention the need to adjust the dosage of CYP1A2-metabolized drugs after smoking cessation, this is recommended in clinical practice, especially for drugs with a narrow therapeutic index. Particularly, increased plasma concentrations of these drugs after smoking cessation may cause serious clinical consequences [16]. Due to the short turnover time of CYP1A2, empirical dose reduction may be necessary within two to three days after smoking cessation.

Because both CYP1A2 expression and activity reductions have been shown to be comparable in quitters and those switching to THS 2.2 due to reduction in exposure to PAHs [6], the same recommendations for dosage adjustment for CYP1A2-metabolized drugs that guarantee a reduction in the exposure to PAHs should be made upon switching to RRPs.

Conclusions

Taken together, these results demonstrate an overall reduced impact on xenobiotic metabolism upon exposure to THS 2.2 aerosol compared with CS in non-clinical *in vitro* and *in vivo* studies as well as clinical studies.

Switching to RRPs reduces CYP1A2 activity to a similar extent as smoking cessation. The same recommendations for dose modification made for smokers upon cessation should be extrapolated to smokers switching to THS 2.2 or other RRPs.

End-users and quitters should be made aware of any unwanted effects associated with smoking cessation that might affect a smoker's attempt to quit as well as the potential effects of switching to RRPs.

References

1. Gabriel et al., Quantitative Prediction of Drug Interactions Caused by CYP1A2 Inhibitors and Inducers. Clin Pharmacokinet, 2016. 55(8): p. 977-90.
2. Anderson et al., Pharmacokinetic Drug Interactions with Tobacco, Cannabinoids and Smoking Cessation Products. Clin Pharmacokinet, 2016. 55(11): p. 1353-1368.
3. Tantcheva-Poor et al., Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. Pharmacogenetics, 1999. 9(2): p. 131-44.
4. Faber et al., Time response of cytochrome P450 1A2 activity on cessation of heavy smoking. Clin Pharmacol Ther, 2004. 76(2): p. 178-84.
5. FDA (Food and Drug Administration), Guidance for industry - Modified risk tobacco product applications - Draft Guidance. 2012.
6. Smith et al., Evaluation of the Tobacco Heating System 2.2. Part 1: Description of the system and the scientific assessment program. Regul Toxicol Pharmacol, 2016. 81 Suppl 2: p. S17-S26.
7. Phillips et al., A Six-Month Systems Toxicology Inhalation/Cessation Study in ApoE-/- Mice to Investigate Cardiovascular and Respiratory Exposure Effects of Modified Risk Tobacco Products, CHTP 1.2 and THS 2.2, Compared with Conventional Cigarettes. Food Chem Toxicol. 2019 Feb 11. pii: S0278-6915(19)30058-4
8.Haziza et al., Assessment of the reduction in levels of exposure to harmful and potentially harmful constituents in Japanese subjects using a novel tobacco heating system compared with conventional cigarettes and smoking abstinence: A randomized controlled study in confinement. Regul Toxicol Pharmacol, 2016. 81: p. 489-499.
9. Haziza et al., Evaluation of the Tobacco Heating System 2.2. Part 8: 5-Day randomized reduced exposure clinical study in Poland. Regul Toxicol Pharmacol, 2016. 81 Suppl 2: p. S139-S150.
10. Ludicke et al., Effects of Switching to the Menthol Tobacco Heating System 2.2, Smoking Abstinence, or Continued Cigarette Smoking on Clinically Relevant Risk Markers: A Randomized, Controlled, Open-Label, Multicenter Study in Sequential Confinement and Ambulatory Settings (Part 2). Nicotine Tob Res, 2018. 20(2): p. 173-182.
11. Ludicke et al., Effects of Switching to the Tobacco Heating System 2.2 Menthol, Smoking Abstinence, or Continued Cigarette Smoking on Biomarkers of Exposure: A Randomized, Controlled, Open-Label, Multicenter Study in Sequential Confinement and Ambulatory Settings (Part 1). Nicotine Tob Res, 2018. 20(2): p. 161-172.
12. Haziza et al., Reduction in Exposure to Selected Harmful and Potentially Harmful Constituents Approaching those Observed Upon Smoking Abstinence in Smokers Switching to the Menthol Tobacco Heating System 2.2 for Three Months (Part 1). 2018.
13. Iskandar et al., 3-D nasal cultures: Systems toxicological assessment of a candidate modified-risk tobacco product. ALTEX. 2017;34(1):23-48.
14. Phillips 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. Toxicol Sci, 2016. 149(2): p. 411-432.
15. Lo Sasso G et al., 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, Early Online: 1–15, 2016.
16. Schaffer et al., A review of smoking cessation: potentially risky effects on prescribed medications. J Clin Nurs, 2009. 18(11): p. 1533-40.

SOT 2019, Baltimore, USA | March 10-14, 2019

Competing Financial Interest – The research described in this poster was sponsored by Philip Morris International.