Biomarkers of Potential Harm in Smoking Abstinence and in the Use of Vuse Electronic Nicotine Delivery Systems (ENDS)

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Abstract

Qualified Biomarkers of Potential Harm (BoPH) are useful in evaluating the beneficial effects of abstinence from cigarette smoking or switching to potentially reduced risk tobacco products. We benchmarked BoPH changes in a 14-Day smoking abstinence (SAB) study in two age groups and used those BoPH to assess the effects of Vuse electronic nicotine delivery systems (ENDS) products. This SAB study was conducted under confinement conditions, and enrolled 70 subjects into younger (24-34 years, n=33) and older (35-60 years, n=37) groups. Several biomarkers of exposure (BoE) and BoPH were evaluated.

Significant declines in Leukotriene E4 (LTE4), 2,3-dinor-thromboxane B2 (2,3-d-TXB2), neutrophils, white blood cells (WBC) and select arterial blood gas [ABG] parameters were observed in both age groups at Days 7 and 14 compared to baseline, while other BoPH, (e.g., ABG, Fractional Exhaled Nitric Oxide [FeNO]) showed age-related effects.

In a separate confinement study where smokers abstained from smoking or switched to three Vuse ENDS products for 7 days, complete blood counts were analyzed. The results showed that WBC, neutrophil and red blood cell (RBC) counts, along with hematocrit and hemoglobin levels decreased in smokers who were switched to Vuse ENDS to the same extent observed in 7 days of smoking abstinence. The BoPH assessed in these studies are indicators of platelet activation (2,3-d-TXB2) and inflammation (LTE4, WBC and neutrophils), and provide useful clinical risk markers for assessing candidate modified risk tobacco products in short-term studies. In each study, rapid and reproducible reductions in LTE4, 2,3-d-TXB2, WBC and neutrophil counts were consistently detected following smoking abstinence or switching to Vuse ENDS, indicating the value of these markers as BoPH.

Study Objectives

- The primary objective of the SAB study was to determine the levels of LTE4 and 2,3-d-TXB2 in the two age cohorts during two weeks of smoking abstinence
- Assess select BoPH in smokers who switched to Vuse ENDS

Methods

Ethical Conduct: SAB study and Vuse ENDS study were approved by a fully accredited Institutional Review Board and were conducted under the principles of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP).

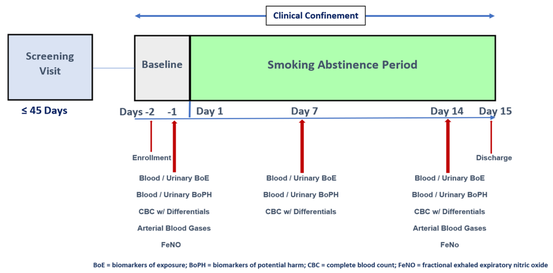
Smoking Abstinence Study Design: Single-center, two-cohort, smoking abstinence study, in which generally healthy adult male and female smokers participated. Smokers of 10-30 cigarettes per day for at least 5 years prior to screening were recruited. A total of 70 subjects (51 males, 19 females) were enrolled between the two age cohorts (24-34 age cohort, n=33; 35-60 age cohort, n=37).

Vuse ENDS Study Design: Two-center, randomized, controlled, open-label, parallel cohort design of an in-clinic switch from non-menthol combustible cigarettes (CC) to one of three Vuse ENDS investigational products (IPs) or smoking abstinence.

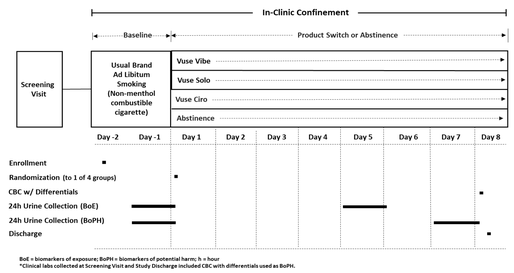
Statistical Analysis: A two-sided paired t-test was used to assess the statistical significance of changes from baseline to Day 7 or Day 14. All statistical analyses were performed using SAS (Cary, NC) and statistical significance was considered at $p \leq 0.05$.

Methods

Figure 1 and 2: Study Schematics of Smoking Abstinence and Vuse ENDS Studies



Smoking Abstinence Study



Vuse ENDS Study

Figure 1: The smoking abstinence study consisted of a 14-day clinical confinement of smokers to evaluate biomarker changes. Blood and urine samples were collected at baseline (Day -1) and Days 7 and 14 of smoking abstinence. Biomarker measurements were made at baseline, 7 days and/or 14 days of smoking abstinence as indicated.

Figure 2: The Vuse ENDS Study was a 7-day product switching study. In this study, adult smokers smoked their usual brand cigarettes *ad libitum* for two days and were then randomized to one of three Vuse ENDS products for a 7-day *ad libitum* use period or smoking abstinence. The ENDS products (Vuse Solo, Vuse Ciro, Vuse Vibe) are marketed by R.J. Reynolds Vapor Company. This study utilized the CBC with differential data collected as part of safety assessments.

Results

Figure 3. Summary of % Changes from Baseline (Day -1) in Urinary (A) and Blood (B) Biomarkers of Exposure on Days 7 and 14 of Smoking Abstinence

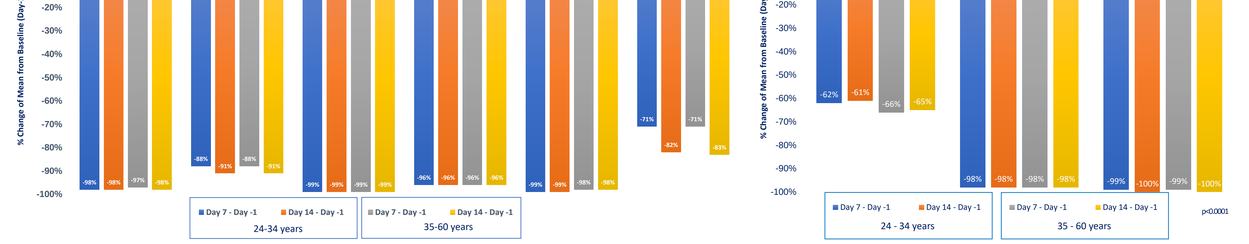


Figure 3. Total nicotine equivalents (NicEq-T), a composite measure of the excretion of nicotine plus five metabolites, declined from baseline on both Days 7 and 14 (about 98% reduction) in both age cohorts. The 2-cyanoethylmercapturic acid (CEMA), a representative BoE for volatile organic compounds, markedly decreased from baseline on Day 7 (88%) and Day 14 (91%) in both age cohorts. The urinary levels of the Tobacco Specific Nitrosamines (TSNAs) also rapidly declined, reflective of their half-lives. NNAL-T (a biomarker for NNK exposure) reductions were 71% and 83% on Days 7 and 14, respectively, for both age cohorts. NNN-T levels decreased rapidly in both age cohorts on Days 7 and 14, with a 99% change from the baseline values. Similarly, notable declines in NAB-T and NAT-T levels were observed for both age cohorts on both days. The levels of plasma nicotine and cotinine demonstrated a ≥ 98% reduction for both age cohorts on both Days 7 and 14. Mean Blood COHb levels for both age cohorts were approximately 60% lower on Days 7 and 14 relative to baseline values.
All reductions were significant at p<0.0001.

Figure 4. 2,3-dinor-thromboxane B2 and Leukotriene E4 Levels Rapidly Decline Following Smoking Abstinence

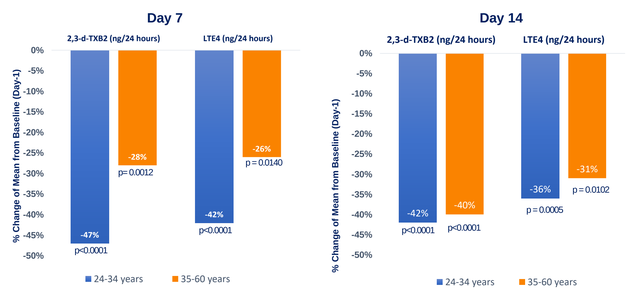


Figure 4: Urinary 2,3-d-TXB2 levels 7 days after abstinence decreased approximately 47% and 28% for the younger and older age cohorts, respectively. Continued abstinence for 14 days sustained the decrease -to about 40% for both cohorts, suggesting a reversal of platelet activation.
Urinary levels of LTE4 were also reduced after 7 days of smoking abstinence by approximately 42% and 26% compared to baseline for the younger and older cohorts, respectively. Similar reductions of approximately 36% and 31% in the younger and older cohorts, respectively, were also observed at 14 days.

Table 1. Hematological BoPH Changes upon Smoking Abstinence Study and Vuse ENDS Study

Hematological Biomarkers		Study Group (Percent Change (p-value*)						
		Smoking Abstinence Study		Vuse ENDS Study				
Biomarker (Units)	Time Point	24-34 years	35-60 years	Time Point	Abstinence	Vuse Solo	Vuse Ciro	Vuse Vibe
White blood cells (10^9/L)	Day 7 vs. Day -2 Day 14 vs. Day -2	-13% (<0.0014) -11% (0.0077)	-25% (<0.0001) -22% (<0.0001)	Day 7 vs. Day -1	-8% (0.0328)	-10% (0.0046)	-9% (0.0093)	-11% (0.0025)
Neutrophils (10^9/L)	Day 7 vs. Day -2 Day 14 vs. Day -2	-18% (<0.0001) -17% (0.0004)	-31% (<0.0001) -28% (<0.0001)	Day 7 vs. Day -1	-15% (0.0033)	-16% (0.0018)	-18% (0.0004)	-17% (0.0004)
Lymphocytes (10^9/L)	Day 7 vs. Day -2 Day 14 vs. Day -2	-5% (0.1201) -1% (0.6331)	-16% (<0.0001) -11% (0.003)	Day 7 vs. Day -1	-2% (0.5372)	-3% (0.3144)	0% (0.9743)	-4% (0.2635)
Red blood cells (10^{12}/L)	Day 7 vs. Day -2 Day 14 vs. Day -2	-2% (0.0147) -4% (<0.0001)	-3% (0.0038) -5% (<0.0001)	Day 7 vs. Day -1	-4% (0.0352)	-4% (<0.0001)	-4% (<0.0001)	-3% (0.0027)
Hematocrit (%)	Day 7 vs. Day -2 Day 14 vs. Day -2	-2% (0.0163) -4% (<0.0001)	-3% (0.0007) -4% (<0.0001)	Day 7 vs. Day -1	-5% (0.0156)	-4% (<0.0001)	-4% (<0.0001)	-3% (0.0014)
Hemoglobin (g/dL)	Day 7 vs. Day -2 Day 14 vs. Day -2	-3% (0.0117) -4% (<0.0001)	-3% (0.0003) -4% (<0.0001)	Day 7 vs. Day -1	-4% (0.0402)	-5% (<0.0001)	-5% (<0.0001)	-3% (0.0009)

Table 1. WBC, neutrophil counts were significantly lower after 7 days (18% and 31% decline) and 14 days (17% and 28% decline) of abstinence in both younger and older age cohorts, respectively. Lymphocyte counts, while lower in both age cohorts, only reached statistical significance in the older age cohort at Day 7 (16%) and Day 14 (11%). Smoking abstinence for 7 days resulted in decreased levels of RBC (2% and 3%), hematocrit (2% and 3%) and hemoglobin (3% and 3%) in the younger and older age cohorts, respectively in smoking abstinence study. At 14 days of abstinence, further declines in RBC counts, hematocrit and hemoglobin (4% for all parameters) in both age cohorts were observed. In Vuse ENDS study, both WBC and neutrophil counts were statistically significantly lower in smokers who switched to Vuse ENDS, and these declines were comparable to the smoking abstinence group. The declines in WBC and neutrophil counts, respectively, were 10% and 16% in Vuse Solo, 9% and 18% in Vuse Ciro, and 11% and 17% in Vuse Vibe. A reduction in the RBC measures was also evident in smokers switched to the three Vuse ENDS products, with declines ranging from 3-4%, comparable to the abstinence group.

Results Cont'd

Figure 5. Changes in Arterial Blood Gases and Exhaled Nitric Oxide upon Smoking Abstinence

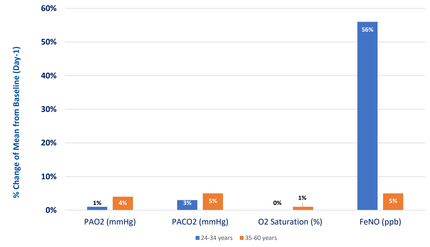


Figure 5: Statistically significant changes in the mean differences from baseline for each cohort were found in several of the ABG indices on Day 14 relative to baseline values: The partial pressure of oxygen (PAO2) saw a 4% increase from baseline; the partial pressure of CO2 (PACO2) mean differences from baseline for the younger cohort and the older cohort increased 3% and 5%, respectively; percent oxygen (O2) saturation was 1% higher from baseline -for the older cohort. Overall, though the change is smaller, the older age group showed significant improvement compared to the younger age group. For FeNO, a marked increase from baseline was observed for only the younger cohort (reflecting a 56% improvement; p<0.05) on Day 14.

Discussion

- This SAB study demonstrated significant reductions in 2,3-d-TXB2 and LTE4 levels in both age cohorts following 7 days and 14 days of smoking abstinence
- For the urinary and blood BoE, rapid, significant reductions were seen by Day 7 and were maintained or further reduced through Day 14 in both age cohorts
- Small, but significant differences, in ABG indices were observed in both age cohorts
- Younger cohort showed a stronger improvement in FeNO compared to older cohort.
- Significant reduction in WBC and neutrophils were observed in the both the SAB study and Vuse ENDS study. Change in WBC are relatable to reduction in neutrophil subset.

Conclusions

- Taken together with previous findings, 2,3-d-TXB2 and LTE4 are useful short-term BoPH in assessing smoking abstinence or switching to non-combustible tobacco products.
- WBC, neutrophils and FeNO could be potential biomarkers in short-term tobacco and nicotine studies.
- Smokers who switched to abstinence or exclusive use of Vuse ENDS products experience rapid improvements in BoPH indicative of platelet function, airway hypersensitivity and inflammation. Thus, some BoPH changes involved in the progression of smoking-induced diseases appear to reverse rapidly upon smoking abstinence and switching to the Vuse ENDS products.

References

Makena et al., 2019. Cancer Epidemiol Biomarkers Prev, 28:2095-105