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

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A BETTER TOMORROW

Biomarkers of Potential Harm (BoPH): Background

- BoPH
  - The “measurement of an effect due to exposure”
- Need for BoPH
  - Early indicators of physiologic changes
  - Useful for product evaluations
    - Modified risk
    - Appropriate for the protection of the public health
- Existing BoPH
  - Forced Expiratory Volume in 1 second (FEV1), White Blood Cell (WBC) count, HDL Cholesterol
- Need short-term BoPH for product switching studies
  - To detect early biological changes
  - Relationship between Biomarkers of Exposure (BoE) and BoPH

Smoking Abstinence Study: Background and Study Rationale

- Study Background
  - Previous RAIS studies qualified two arachidonic acid (AA) metabolites, 2,3-d-TXB2 and LTE4, as short-term BoPH in cross-sectional and product switching studies
  - 2,3-d-TXB2 and LTE4 are linked to platelet activation and inflammation, respectively, and represent BoPH in short-term tobacco product switching studies

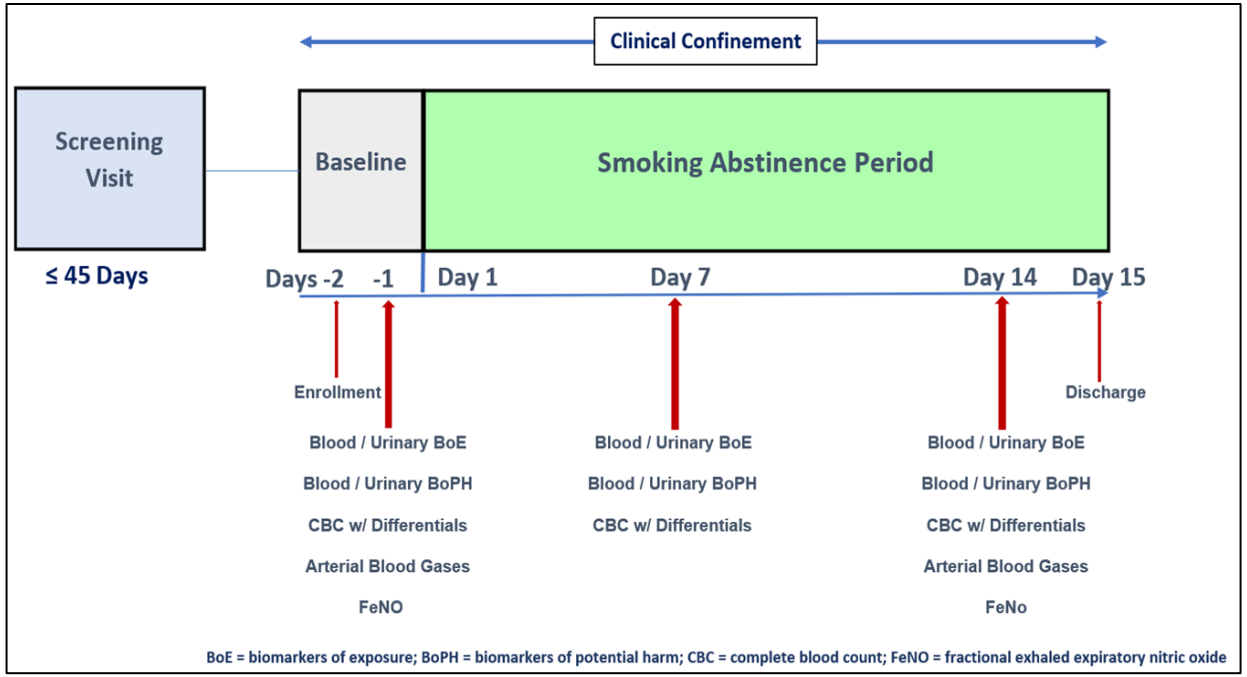
- Study Rationale
  - To further qualify LTE4 and 2,3-d-TXB2 and identify additional BoPH in a two-week smoking abstinence (SAB) study
  - Given that age and duration of chronic smoking could impact BoPH differentially, we assessed the effect of smokers' age on biomarker changes upon smoking abstinence

Study Design: Smoking Abstinence and Vuse ENDS Studies

Smoking Abstinence Study: Single-center, two-cohort, smoking abstinence study, in which generally healthy adult male and female smokers participated.

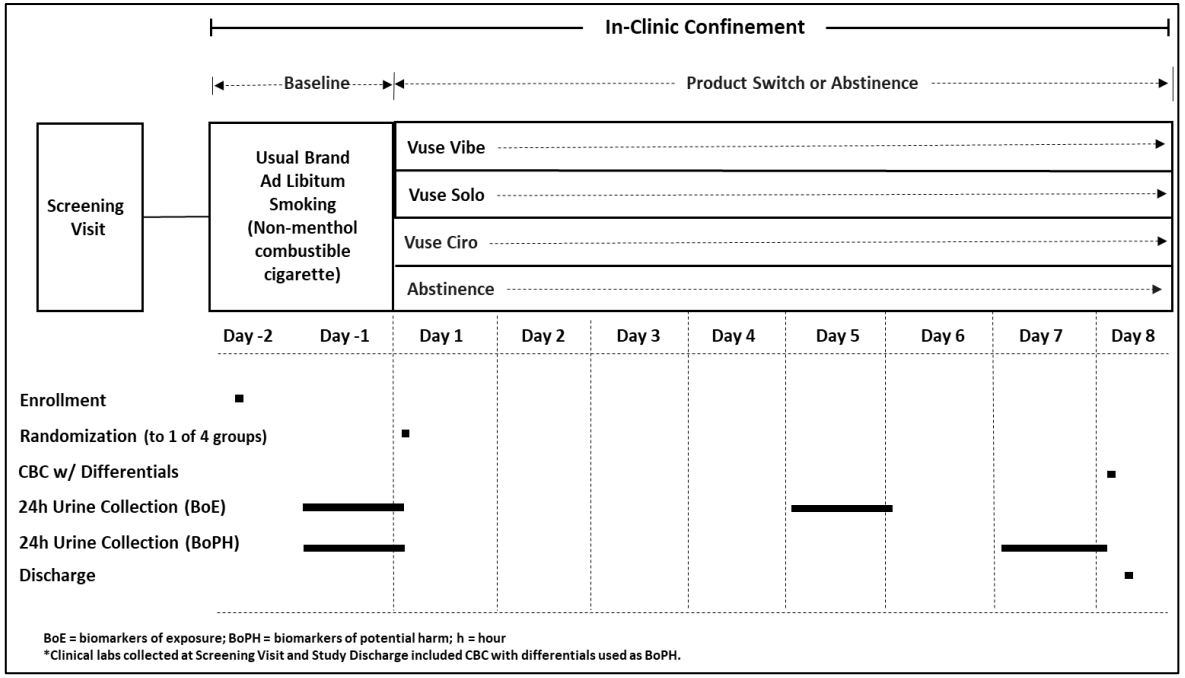
Vuse ENDS Study: Two-center, randomized, controlled, open-label, parallel cohort design of an in-clinic switch from non-menthol combustible cigarettes to one of three Vuse ENDS investigational products or smoking abstinence.

Smoking Abstinence Study



BoE = biomarkers of exposure; BoPH = biomarkers of potential harm; CBC = complete blood count; FeNO = fractional exhaled expiratory nitric oxide

Vuse ENDS Study



BoE = biomarkers of exposure; BoPH = biomarkers of potential harm; h = hour
*Clinical labs collected at Screening Visit and Study Discharge included CBC with differentials used as BoPH.

Smoking Abstinence Study: Objectives and Endpoints

Primary Objective

To measure changes in the Arachidonic acid (AA) metabolites 2,3-dinor-Thromboxane B2 (2,3-d-TXB2), and Leukotriene E4 (LTE4) as BoPH in two cohorts of smokers (ages 24-34 years and 35-60 years) after 14 days of smoking abstinence

Primary Endpoints

Biomarkers of Potential Harm (Days -1 and 14 only)

- AA metabolites, 2,3-d-TXB2 and LTE4

Secondary Objectives

To measure changes in AA metabolites 2,3-d-TXB2 and LTE4 after 7 days of smoking abstinence

To measure changes in additional BoPH and BoE after 7 and 14 days of smoking abstinence

Secondary Endpoints

Urinary Biomarkers of Exposure (Days -1, 7, and 14)

- Total nicotine equivalents (molar sum of nicotine + 5 metabolites)
- 2-cyanoethylmercapturic acid (CEMA)
- Tobacco Specific Nitrosamine(s) (TSNAs)

Blood Biomarkers of Exposure (Days -1, 7, and 14)

- Whole blood carboxyhemoglobin (COHb)
- Plasma nicotine
- Plasma cotinine

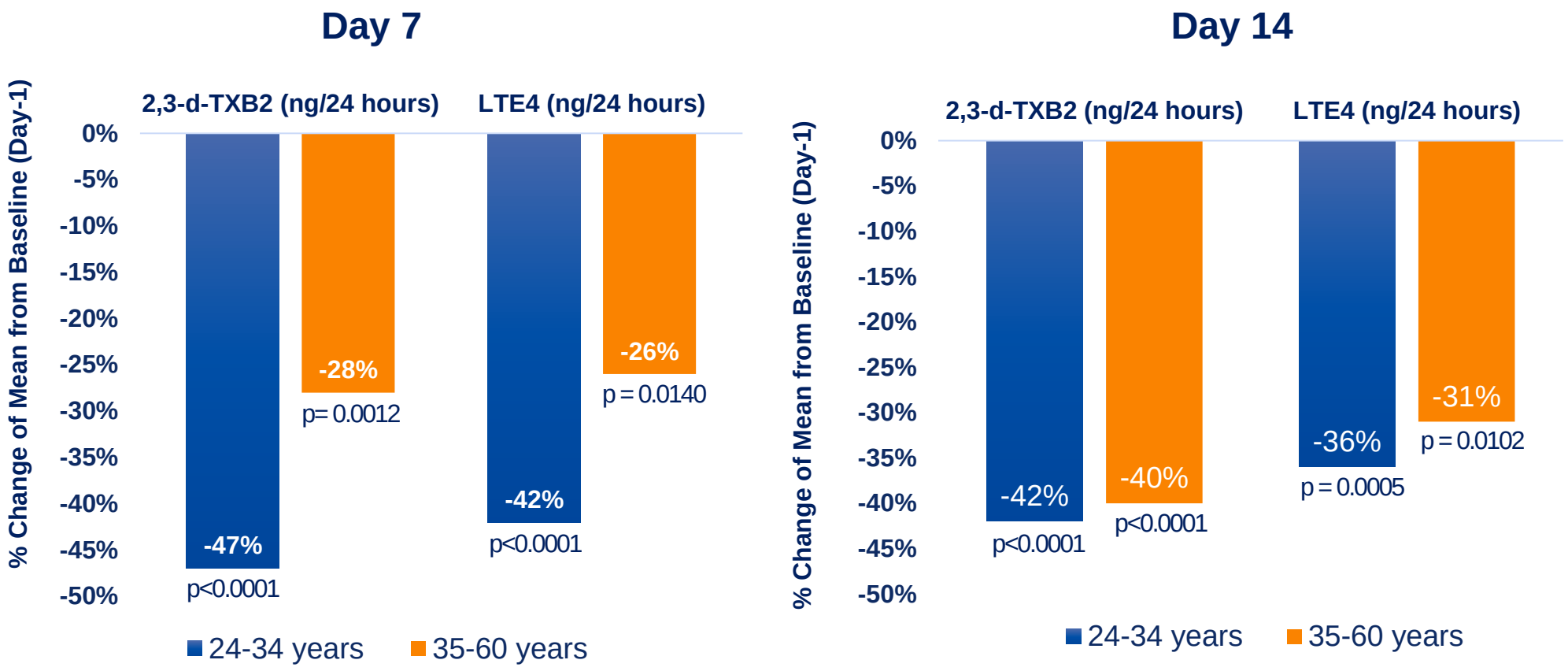
Biomarkers of Potential Harm – Biochemical (Days -1, 7, and 14)

- Arachidonic acid metabolites (including TxM & LTE4 on Day 7)

Biomarkers of Potential Harm – Physiological (Days -1 and 14)

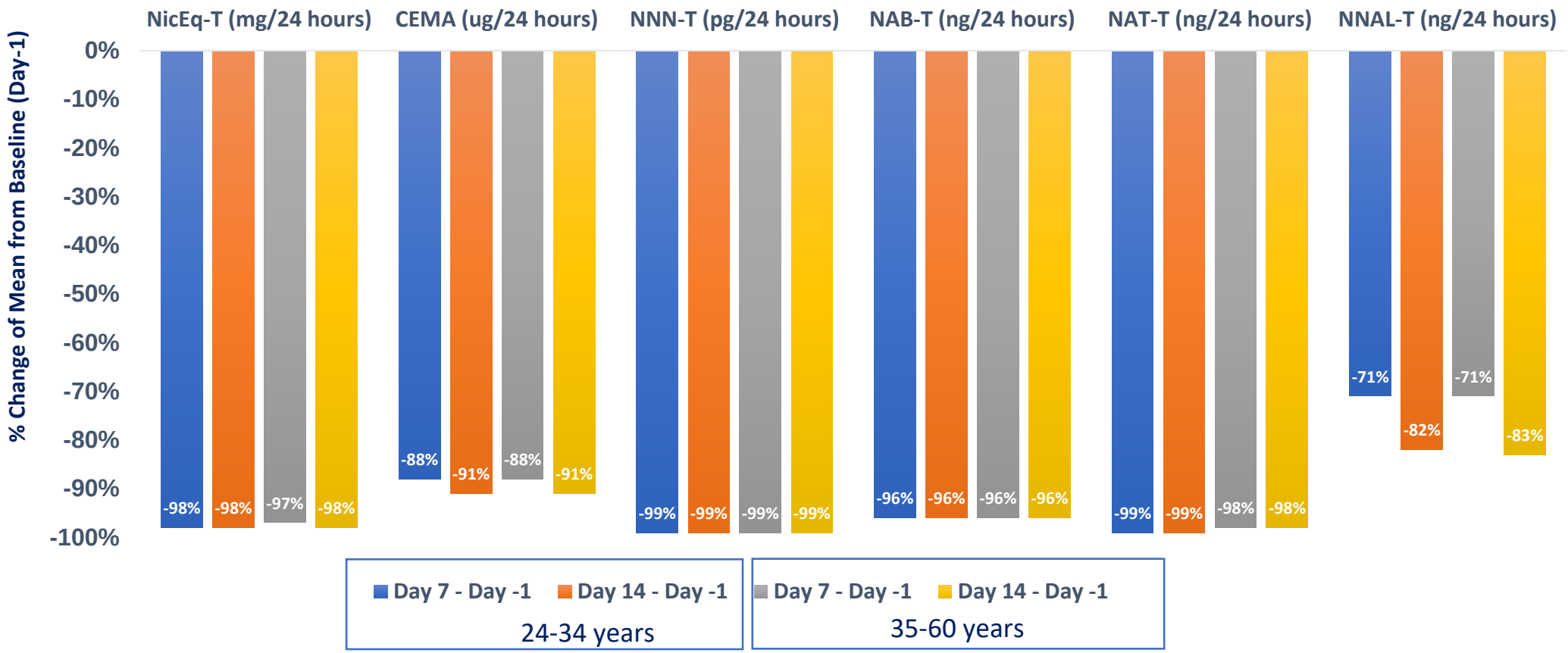
- Fractional expired nitric oxide (FeNO)
- Arterial blood gases (ABG)

2,3-dinor-Thromboxane B2 and Leukotriene E4 Levels Rapidly Decline Following Smoking Abstinence



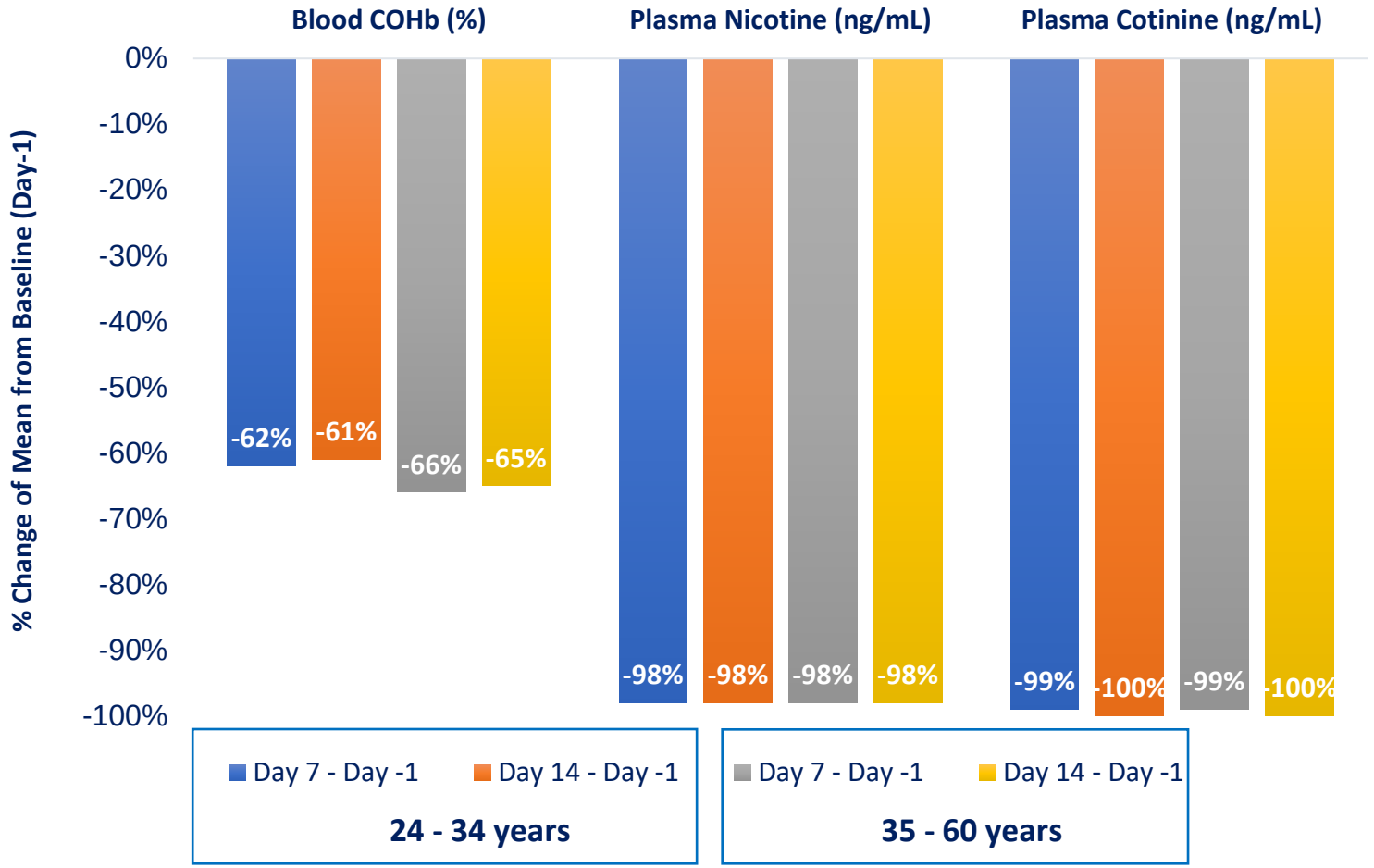
P-values represent the statistical significance of the mean differences from baseline (Day -1)

Urinary Biomarkers of Exposure Levels Rapidly Decline Following Smoking Abstinence



All changes in biomarkers where statistically significantly different ($p<0.0001$) from baseline at both Day 7 and Day 14

Blood Biomarkers of Exposure Levels Decline Following Smoking Abstinence

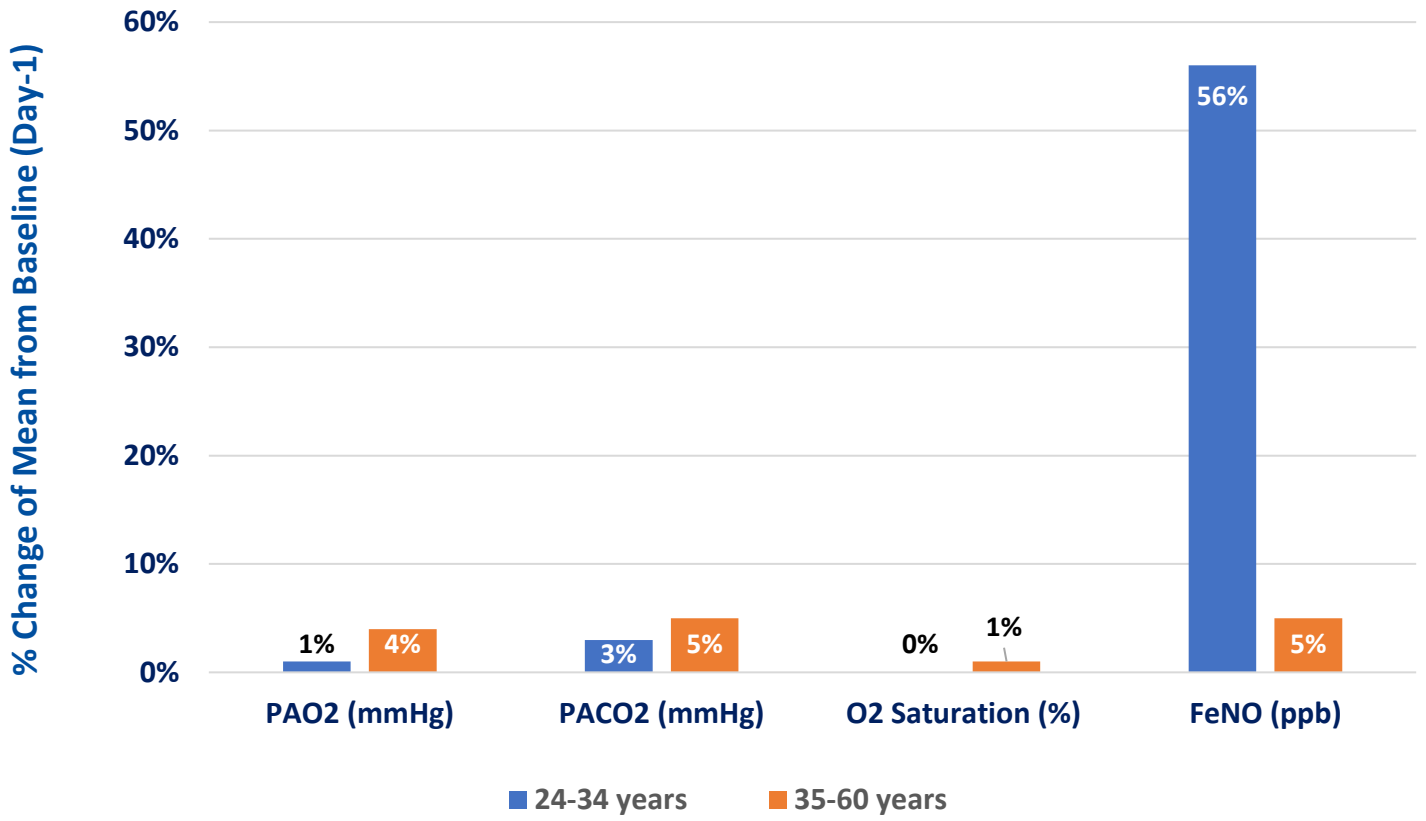


All changes in biomarkers where statistically significantly different ($p<0.0001$) from baseline at both Day 7 and Day 14.

Decline in Inflammatory BoPH upon Smoking Abstinence and Vuse ENDS Use

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)

Improvements in Arterial Blood Gases (ABG) and Exhaled Nitric Oxide (FeNO) upon Smoking Abstinence



- For ABG, though the change is smaller, the older age group showed significant improvement compared to the younger age group.
- For FeNO, significant improvement was observed in younger cohort.

Summary

- This Smoking Abstinence (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
- Statistically significant reductions in almost all hematological markers were observed in both the SAB and Vuse ENDS studies.
  - Changes in WBC are relatable to reduction in the neutrophil subset
- Changes in lymphocytes were not statistically significantly different following smoking abstinence or switching to Vuse.

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 research studies.
- Switching from combustible to ENDS or smoking abstinence had the same biological outcomes

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