**Biomarkers of Exposure and Effect in the Lungs of Smokers, Non-Smokers and Electronic Cigarette Users**

**Online Methods section**

## **Participants**

This study underwent concept-review by the National Cancer Institute, the Food and Drug Administration, and the OSU Tobacco Center of Regulatory Science. The Ohio State University (OSU) Clinical Science Research Committee and the Institutional Review Board approved the study protocol. The study was also peer-reviewed by the Ohio State University Comprehensive Cancer Center Intramural Research Program, which includes external peer review. It also has been approved and monitored by an external Data Safety and Monitoring Board. Subjects received $200 for the procedure.

Healthy never-smokers (21-30 years old) were recruited between 2015 and 2017 using a variety of advertising methods, namely The OSU Study Search website, Research Match website, a participant registry from the OSU Tobacco Centers of Regulatory Science, local print and television media, and Craig’s List. Eligibility criteria included never-smokers who had smoked less than 100 cigarettes in their lifetime, as defined by the Centers for Disease Control (<https://www.cdc.gov/tobacco/data_statistics/fact_sheets/adult_data/cig_smoking/index.htm>), and not for at least 1 year, and had not used an e-cig for at least 1 year prior to enrollment. Lack of regular smoking was confirmed by salivary cotinine using a NicAlert kit (Nymox Pharmaceutical Corporation, St. Laurent, QC, Canada) (never-smokers were defined at test level = 0). Subjects were excluded if they reported having an immune system disorder requiring medication, pulmonary diseases (e.g., asthma within the prior 5 years, acute bronchitis within 1 year, COPD, chronic bronchitis, and restrictive lung diseases), kidney or liver diseases, or other medical disorders that would increase the risk from bronchoscopy, or affect biomarker data. Participants were also excluded if they had general anesthesia during the prior 12 months; used any inhalant medications; had allergies to study medications (lidocaine, Cetacaine, Versed, or Fentanyl); underwent a bronchoscopy or any other lung procedure within the prior 12 months; reported regular marijuana use in the past (>10 times); reported smoking marijuana 3 months prior to the study procedure; reported other combustible tobacco use >10 times; reported other combustible use within 3 months prior to the procedure; or, were pregnant (determined by urine pregnancy prior to bronchoscopy). All subjects provided written informed consent.