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**Scientific Abstract**

Smoking is an independent risk factor for the development of coronary artery disease, abdominal aortic aneurysm (AAA) and other lesions in the circulatory system. These complications are a significant cause of morbidity and mortality worldwide, causing deaths by heart attack and stroke that exceed deaths by tobacco-related cancers. In the vasculature, tobacco compounds damage vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs), often resulting in life-threatening consequences for the affected patient. We propose to establish methodology to screen many chemical components in tobacco smoke on VECs and VSMCs and determine the effects of these chemicals on genetically susceptible individuals. As the effect of tobacco products on the integrity of the vasculature differs among individuals, we presume genetic variations contribute to patient outcome. However, the genetic components related to tobacco-induced vascular disease susceptibility are unknown. The identification of molecular biomarkers and related signaling pathways is essential for risk stratifications of smokers and the identification of distinguishing adverse effects of different tobacco compounds on human health. We propose to generate induced pluripotent stem cell (IPSC) lines from 20 NIH Clinical Center patients who smoke (Boehm NHLBI). Patients will be identified, categorized and enrolled into a clinical protocol that allows IPSC line generation (Chen, Arai NHLBI). We will develop a high-throughput compound screen that includes 20 tobacco related substances, including the additive menthol, in an 8-dose range of each chemical, and a 1-5 biomarker assay endpoint using 20 patient specific iPSC-derived EC and VSMC lines (Gerhold, McKew NCATS). Obtained data will be processed and evaluated by NCATS group and shared with collaborators and made publically available. Subsequent investigations will clarify pathway-compound-biomarker interactions (Gerhold, McKew NCATS; Finkel, Boehm NHLBI).
The primary hypothesis-generating screen will contain 10 patient-specific lines from smokers without vascular disease and 10 patient-specific iPSC lines from smokers with severe vascular disease. A second verification screen will contain 10 patient-specific IPSC lines from smokers unrelated to the initial patient population of first screen, and investigators will be blinded to the presence of vascular disease in this second population. This project will identify new biomarkers to distinguish the impact of different tobacco products on vascular disease. Patient-specific lines will be genetically engineered to facilitate large-scale cell lineage differentiation into VECs and VSMCs and contain reporter systems necessary to develop an iPSC-based quantitative high throughput compound screening (qHTS) (Boehm NHLBI, Rao NIHCRM NIAMS). Both the unmodified and engineered cell lines will be available for academic and commercial usage.