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Headline: Multiomics Approaches to Biomarker Discovery in Cardiovascular Disease: Smoking, Cessation, and Reduced-Risk-Products

Byline: Carine Poussin, PhD, Philip Morris International (PMI)

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Smoking-related diseases are caused by long-term exposure to the toxicants found in cigarette smoke. Novel techniques in systems biology, combining -omics analyses with phenotypical and functional endpoints, allow for the investigation of the biological networks that drive the progression of these diseases at the molecular level. They can be used to understand how these biological networks are perturbed by smoking and the extent to which the perturbations are reversible with regard to smoking cessation. In turn, these novel techniques may prove useful for the identification of smoking-related biomarkers of exposure that could be included in strategies to test novel reduced-risk products*, such as e-cigarettes and heat-not-burn tobacco products, and assess their ability to reduce smoking-related health risks in smokers who switch to them.

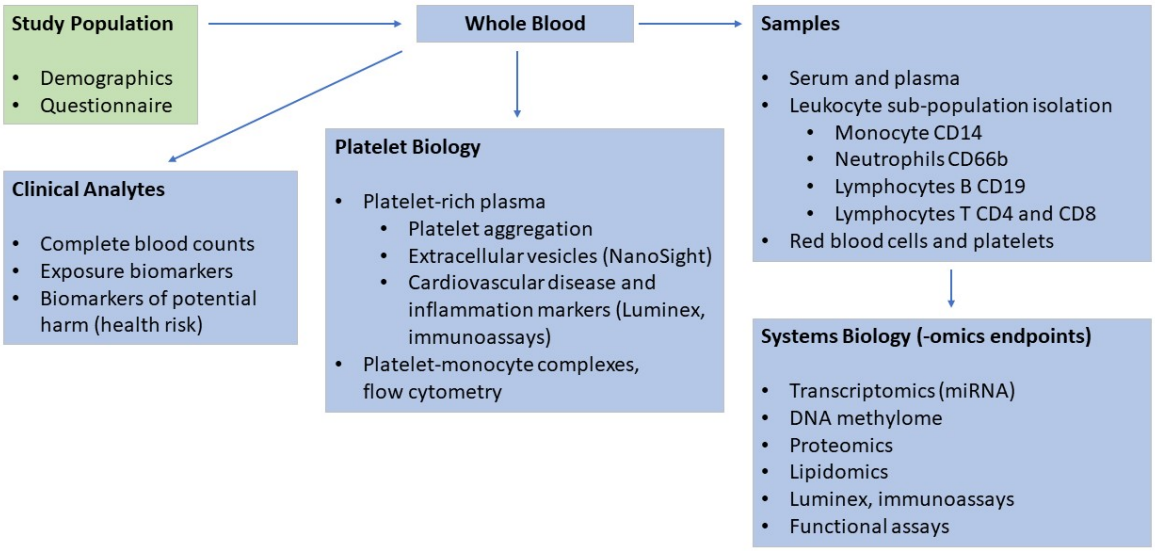
At the recent European Atherosclerosis Society annual meeting in Lisbon, Portugal, Philip Morris International (PMI) scientists presented posters of two studies that make extensive use of systems biology methods. One ongoing clinical study is designed to elucidate the molecular effects of smoking and smoking cessation and to understand the impact of e-cigarette vaping.[1] Its aim is to discover predictive models of smoking-related cardiovascular health effects that can be leveraged in future research. The second study combined -omics, physiological and histological endpoints in an in vivo systems toxicological analysis of the cardiovascular and respiratory effects of two candidate reduced-risk products in a six-month inhalation study.[2]

Smoking and Smoking Cessation

The first study, being conducted in collaboration with the University of Rochester Medical Centre (URMC), collects circulating blood cells from smokers, former smokers, e-cigarette users, and individuals who have never smoked. Investigations on platelet biology in these groups are also conducted. Human volunteers are recruited and provide consent in accordance with the Helsinki declaration under a protocol approved by the University of Rochester Institutional Review Board.[3] Recruitment is currently ~80% complete. Individuals must be between 21 and 55 years old with specific inclusion criteria for consumption habits and history of smoking, smoking cessation and e-cigarette vaping, and with exclusion criteria including pregnancy, recent use of medications, acute illness, and certain defined pathologies. The investigations of smoke and e-cigarette vapour exposure on human blood cells involve transcriptomics, lipidomics, and proteomics, as well as measures of inflammation (eg, cytokine

production), oxidative stress (eg, DNA methylation and monoamine oxidase activity), and clinical endpoints to identify critical biomarkers and specific molecular signatures.

Study Schematic



Concentrations of nicotine and nicotine metabolites are measured by URMC Clinical Laboratories. As would be expected, the concentrations of both are significantly higher in smokers as compared to never smokers or former smokers. These measures provide quantitative estimates of the uptake of selective smoke compounds by the different groups in the study population. URMC Clinical Laboratories also determine the concentrations of a panel of biomarkers of potential harm. Changes in levels of these biomarkers may be associated with increased health risks, with results so far indicating that differences identified between the study groups reflect an increase of systemic inflammation in smokers. Flow cytometry is used to measure platelet monocyte complexes, a marker of increased levels of vascular inflammation, which have been found to be significantly higher in smokers as compared to never smokers in the preliminary analysis.

Further analyses will examine large-scale -omics data alongside demographic and smoking history variations to identify mechanisms and markers of vascular dysregulation that are associated with smoking, and to understand the impact of smoking cessation and e-cigarette vaping on them.

Reduced-Risk Products

The second study presented at the European Atherosclerosis Society used -omics analyses to investigate the cardiovascular and respiratory effects of PMI's Tobacco Heating System 2.2 (THS 2.2) and Carbon Heated Tobacco Product 1.2 (CHTP 1.2). In both THS 2.2 and CHTP 1.2, tobacco is heated rather than burned, resulting in significantly reduced levels of harmful chemicals emitted and inhaled as compared with cigarette smoke. Apoe-/- deficient mice were exposed for six months to either fresh air, CHTP 1.2 aerosol, THS 2.2 aerosol, or cigarette smoke for three hours per day. The impact of smoking cessation or switching from smoking to CHTP 1.2 aerosol exposure was also evaluated. An extensive molecular, high-throughput analysis involving transcriptomics, proteomics, and lipidomics was used to analyse cardiovascular and respiratory effects, alongside physiological and histological endpoints.

Exposure to cigarette smoke resulted in an increase in the number of differentially expressed genes in lung and heart ventricles, in comparison to exposure to fresh air alone. Exposure to

the heat-not-burn aerosols (THS 2.2, CHTP 1.2) did not result in differentially expressed genes in comparison with the fresh air group. In the cessation and switching groups, the number of dysregulated genes was decreased strongly in comparison with those observed following cigarette smoke exposure. Collectively, the study data showed that the biological impact of switching from cigarette smoke exposure to CHTP 1.2 aerosol exposure approaches that of cessation and halts the development of both cardiovascular and respiratory disease endpoints. These findings are in line with those of previous, similar studies looking at THS 2.2.[4,5] Study results were consistent across biologically plausible and relevant mechanisms of disease causation (ie, inflammation, cell stress, cell proliferation, tissue repair and angiogenesis, and cell fate).

The European Atherosclerosis Society Annual Meeting

The European Atherosclerosis Society annual meeting brought together world-leading clinicians and researchers studying atherosclerosis and related vascular disease. Presentations in plenary sessions, focused workshops and advanced clinical seminars highlighted the latest developments in the understanding of the mechanisms of atherosclerosis, as well as therapeutic strategies to prevent, slow down and even regress its progression. The high-level of interdisciplinary exchange during the meeting will help to generate increased levels of understanding of vascular disease within the scientific community.

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* Reduced-Risk Products ("RRPs") is the term we use to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to these products versus continued smoking. We have a range of RRPs in various stages of development, scientific assessment and commercialisation. Because our RRPs do not burn tobacco, they produce far lower quantities of harmful and potentially harmful compounds than found in cigarette smoke.

Where can readers find more information?

Comprehensive information on PMI's Research and Development programmes can be found online at www.pmiscience.com.

About Carine Poussin, PhD

Dr. Carine Poussin leads various computational and experimental research and systems toxicology-based product assessment projects at Philip Morris International. Working for many years in academia and industry, Dr Poussin has gained broad experience in computational biology, data analysis, and interpretation in diverse disease areas ranging from cardiovascular, lung, immune, and metabolic diseases in the context of medical, nutritional and toxicological research. Dr Poussin is also part of the scientific team responsible for the development of sbv IMPROVER computational challenges at Philip Morris International.

References

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