

Epigenetic biomarkers of tobacco smoke exposure PI: Douglas Bell CoPI Jack Taylor, Stephanie London NIEHS; Nat Rothman, Lee Moore, Debra Silverman, NCI. Each have >20 years of research experience studying tobacco-induced biomarkers and disease.

Objective: Using genomic arrays of 473,844 CpG sites on umbilical cord blood samples we have identified specific DNA methylation changes associated with maternal smoking. We have confirmed these findings in adult smokers, thus identifying both a set of epigenetic markers, and mechanistic pathways for transplacental and adult smoking-associated diseases. We propose further validation of these epigenetic marks for use in evaluating the relative toxicity of tobacco products. This validation will examine the dose, time-course and persistence of the epigenetic changes from tobacco products, identify the target cells for epigenetic-mediated change, and characterize toxic effects at the molecular and cellular level. This work responds to Priorities 5, 21, 22, 28, 29 and 30.

Hypothesis: Exposure to tobacco products causes epigenetic reprogramming of hematopoietic stem cells resulting in altered gene expression and cellular response. These effects in blood are biomarkers of tobacco exposure and tobacco-induced disease.

Aim 1. Extend our findings of methylation biomarkers using existing samples from people with well-characterized tobacco-smoking histories where we have made measurements of cotinine, chromosome aberrations, somatic gene mutations, and genetic polymorphisms.

Aim 2. In a prospective cohort study of smoking-induced bladder cancer, test the relationship between tobacco exposure, methylation biomarkers, and future risk of developing tobacco-induced bladder cancer to test the predictive value of the methylation biomarkers among smokers for this tumor.

Aim 3. To determine the best tissue for population monitoring for regulatory purposes, test our methylation biomarker in blood and other tissues from newly-recruited subjects who use various tobacco products. Assess profiles of DNA methylation and identify biomarkers that are in common or unique to a specific exposure. Examine samples from recent quitters to determine the persistence of biomarkers.

Aim 4. To further refine accuracy and specificity of monitoring in blood, we will determine target cells in blood separated into lymphoid, granulocytes, erythroid, and CD34+ cells and compare the relationship between exposure, methylation, and gene expression. We will develop an *in vitro* toxicity assay using cultured blood cells and cell lines and assess methylation as an endpoint in relationship to other toxicity endpoints.

Preliminary: In a Norwegian prospective birth cohort study (n=1062) we identified highly significant ($p < 10^{-8}$) dose-dependent, maternal smoking-induced methylation changes to fetal tissue (revision submitted). Genes involved in hematopoiesis and tobacco smoke metabolism were affected. These unique findings were replicated in a second study in North Carolina and also observed in a small pilot study of adult smokers. We now wish to extend these findings in relationship to other smoking biomarkers, to detect the presence of cell-type specific changes and phenotypic effects, and make connections between tobacco induced epigenetic biomarkers of effect, intermediate molecular effects, and tobacco induced disease outcomes which would support regulatory review of the biomarker.