Center for Novel Biomarkers of Response

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The ultimate goal of the Exposure Biology Program is to understand the development and progression of complex disease by precisely, accurately, and quantitatively assessing the individual's exposure to environmental stressors and the individual's responses to these stressors. Two of the most important risk factors for human morbidity and mortality are exposure to cigarette smoke and obesity; both are associated with systemic chronic inflammation and oxidative stress, which may be the unifying mechanism underlying the development of co-morbidities in cigarette smoke and obesity, as well as for the interaction of these risk factors with other environmental toxicants and the genome. A significant challenge to the use of biomarkers, and to untangling the relationship between complex exogenous and endogenous stressors, is the identification of persistent, mode-of-action-dependent response markers with the requisite sensitivity and selectivity. However, reactive nitrogen species (RNS) and reactive oxygen species (ROS) derived from inflammation and oxidative stress pathways result in modified proteins that can be used as indicators of cellular response to stressors. For example, the changing inflammatory response of the lung, as exposure progresses to disease, offers different chemistries and protein modifications in the form of 3-nitrotyrosine, 3-chlorotyrosine, and 3-bromotyrosine that reflect these differences.

The organizing theme for the PNNL U54 Center for Novel Biomarkers of Response is that identification and validation of persistent modified proteins in plasma will provide specific information about the stressor, its mode of action, and the target organ. The environmental stress of the human and animal projects is focused on main-stream and side-stream cigarette smoke with obesity as a contributing physiological factor. The additional specificity provided by the biosignatures of modified proteins will eventually allow assessment of susceptibility and better elucidate the relationship between genes, exposure, and human disease. Our integrated, multidisciplinary center has three research projects (human, mouse, and sensor) and two technology cores (proteomics and ELISA Microarray) which collectively will have four goals.

Objective 1. Discovery of RNS/ROS-modified peptides as candidate biomarkers with specificity and persistence for environmental stress. We use state-of-the art tandem mass spectrometry to identify the biosignature of peptides modified by reactive nitrogen and reactive oxygen species in plasma from a human population and in parallel experiments in mice. The characterization of modified peptides in human plasma will be performed in samples from 400 participants [non-smokers, smokers, smokers with Chronic Obstructive Pulmonary Disease (COPD)], and side-stream smokers stratified by body mass index. The discovery of modified peptides in mice will be performed in pooled samples from normal and obese mice exposed to main-stream and side-stream cigarette smoke in experiments parallel to the human studies. Two lung- and liver-

specific oxidants, paraquat and carbon tetrachloride, will also be used in the mouse to test the mode-of-action and tissue-dependent response.

Objective 2. Verification of RNS/ROS modified peptides as specific biosignatures for environmental stressors. The biosignature of modified peptides will be verified by data-directed MS/MS based Multiple Reaction Monitoring (MRM).

Objective 3. Validation of RNS/ROS modified proteins for use as specific biomarkers for environmental stressors. The validation of RNS/ROS modifications for use as biomarkers will be performed at the protein level by custom-designed sandwich ELISA microarrays. The validation will include development and evaluation of antibody reagents and assay reproducibility and sensitivity using separate plasma sample subsets for testing and for validation.

Objective 4. Develop, test, and deploy two detector systems for exposure and for both specific and general markers of RNS/ROS response. A laboratory-based ELISA Microarray platform will be used to provide high-throughput, multiplexed analysis of dozens of analytes. The validated biomarkers will be also be deployed on a prototype, clinic-deployable detector system for on-site analysis. The sensor system is nanoparticle-based multiplexed Immunochromatographic / Electrochemical Biosensor (IEB) that will support the measurement of markers for exposure (cotinine) and response, both specific (modified proteins) and generic markers of oxidative stress and chronic inflammation, such as TNF-alpha, in a single platform.

Critical Outcomes and Deliverables. The PNNL U54 Center applies state-of-the-art proteomic and sensor technologies to provide NIEHS with a database of mode-of-action informing biomarkers of response, reagents for selected markers tested and validated in humans and informed by parallel studies in mice. These plasma protein markers will identify response to cigarette smoke, obesity, and to smoking with obesity. The assays will be deployed on (a) a laboratory based ELISA Microarray platform, and (b) on a robust, clinic-deployable nanoparticle-based sensor suitable for use in large-scale human biomonitoring studies to evaluate the interaction of genes and the environment. The results of this U54 Center also will improve the scientific community's ability to compare, contrast and extrapolate biomarkers between humans and mice.