Definition of Genome-wide cell-free DNA fragmentation in patients with oral cancer.

Proposed Investigators:

Ingrid Glurich, Neel Shimpi, Steven Schrodi, Shicheng Guo.

Physician Investigators:

Adedayo Onitilo, MD

Andrew Urquhart (pending agreement-Dr. Urquhart is currently out of office through 10/7/19)

**Abstract:**

**Background:** When identified early, oral cancer is highly treatable with good survival outcomes. However, since reliable biomarkers to detect malignant transformation of potentially malignant oral disorders (OMPD) are currently lacking, oral cavity cancer (OCC) is frequently diagnosed only at later stages associated with poor prognosis, high morbidity and mortality. A recently-defined novel approach by Christiano *et al* (2019) termed ‘DNA evaluation of fragments for early interception (DELPHI) demonstrated capacity to specifically differentiate and classify cancers originating in various organs by genome-wide analysis of cell-free DNA (cfDNA) shed from affected tissue into blood. Based on variability in length of the DNA fragments, unique expression patterns were distinguished for various cancers originating in diverse tissues. Notably, higher quantities of smaller cfDNA were associated with circulating cf tumor DNA. Further, application of machine learning (ML) demonstrated capacity to classify the cancer-specific profile and project the tissue of origin. Detection rate of patients with cancer was over 90%. We propose to apply DELPHI in combination with bisulfite-free epigenetic genome-wide methylation analysis (GWMA) to examine whether analysis of cfDNA can distinguish between metastatic OCC, healthy tissue and OMPD with high fidelity.

**Hypothesis:** DELPHI/GWMA analysis with application of ML can differentiate between healthy oral tissue, tissue impacted by OMPD, and oral cavity cancer.

**Approach:** A prospective pilot study design proposes enrollment and collection of blood from subjects who are: 1) healthy, 2) exhibit OMPD and 3) diagnosed with OCC and paired adjacent normal tissues. Following extraction of cfDNA from plasma, sequencing libraries will be created and subjected to whole genome sequencing and methylation analysis. The ratio of small (100-150 base pairs (bp) to large (151-200 bp) cfDNA fragments distribution and specific position in the genome will be mapped in order to create distinctive profiles associated with healthy oral tissue, OMPD and OCC. Genome mapping will proceed by characterizing cfDNA fragments in five mega-base segments across the length of the genome, generating approximately 20,000 reads/segment at 1-2X genome coverage. Machine learning based deconvolution analysis will be developed to 1) classify oral health status and 2) confirm capacity to identify the oral cavity tissue as the origin of OCC.

**Significance/Impact**: This study will pilot capacity of DELPHI/GWMA to distinguish between OCC, OMPD and healthy oral tissue. Further, this study will identify genomic loci associated with OCC based on definition of genomic segments exhibiting higher ratios of small cfDNA or differential methylation. This novel approach has high translational potential for screening patients at risk for OCC and early detection of malignant transformation. This pilot study will collect preliminary data to demonstrate feasibility of a larger prospective study to validate efficacy of this approach as a potential screening tool for monitoring and detecting early-stage OCC in the clinical setting and serve as process pilot for future clinical trial development. (word count 452)