**BAOMS Endowment Sub-Committee 2017 Grant progress report**

**Project title:** Whole Genome Analysis of Proliferative Verrucous Leukoplakia (PVL)

**Grantee Institution**: University of Liverpool

**Principal Investigator:** Mr Eyituoyo Okoturo

**Mentors:** Professor Richard Shaw, Dr Janet Risk

**Background summary**

A complete biological model is more likely to be uncovered if different levels in genomic, epigenomic and proteomic data are analysed. The aim of the study is to identify genetic alterations in PVL tumour samples by identifying association between phenotypic outcomes and high throughput data measurements like gene expression or epigenetic variations using whole methylome array analysis (MAA) or proteomic and metabolomics studies. My primary objective is to help unravel unreliable, single type data variants which in turn help identify key genomic factors whose interaction predicts biological outcomes

**Hypothesis**

Based on its clinical homogeneity and a comprehensive systematic review, we suggested a single gene pathway aberration that is distinct from the more conventional OED – derived / *denovo* OSCC and to be responsible for its pathogenesis.

**Experimental plan**

**+ *Multi-omic Study Design***

Having earlier carried out a hypothesis generating study of whole exome sequencing (WES) on 5 fresh frozen PVL – derived OSCC and identified candidate genes, a multi-omic data driven design approach was adopted in designing our study samples and generating data for analyses. This comprised:

- **Whole genome sequencing (WGS)**: PVL – derived OSCC x 3
- **Whole methylome array analysis (MAA)**: PVL – derived OSCC x 5, low grade PVL x 2, OED / *denovo* OSCC x 5 and PVL adjacent normal tissue x 3

**+ Laboratory Analysis**

- **WGS**: Genomic DNA was isolated from samples and quantified at an average nanodrop concentration of 50ng/ul. Samples were transferred to the Edinburg Genomic Centre for WGS. The WGS protocol in summary comprised, fragmentation, library construction, elution and purification, adapter ligation for indexing, amplification and binding to flow cell – sequencing on Illumina platform by sequencing by synthesis (SBS). Data is being analysed.
- **MAA**: samples were bisulphite converted using EZ DNA Methylation – Kit. Samples were transferred to Edinburg Genome Centre. The protocol in summary comprised whole genome amplification, enzymatic fragmentation prior to hybridisation to Illumina HumanMethylation BeadChip arrays according to manufacturer's protocol. Arrays were scanned on Illumina's iScan technology. Data is being analysed.

**+ Bioinformatic analysis**

Meta - dimensional analysis by engaging multiple data types in WGS and MAA, towards a multivariate model to attain a given outcome.

Aim of analysis / outcomes
Use repositories of data to explain the following:
* Gene annotations
* Gene expression regulation
* Functional outcomes of gene mutation

**Publications**


1. Okoturo E, Janet Risk, Mark Boyd, Michael Ho, Asterios Triantafyllou, Bijay Rajlawat, Deborah Holt, Richard Shaw. Management of Proliferative Verrucous Leukoplakia: justification for a conservative approach (*Online only article*). British Journal of Oral and Maxillofacial Surgery 2017, Volume 55, Issue 10, Page e69
2. Okoturo E, et al. Molecular pathogenesis of proliferative verrucous leukoplakia: a systematic review. Br J Oral Maxillofac Surg (2017), https://doi.org/10.1016/j.bjoms.2018.08.010


**Other source of funding**

1. **Title**: The molecular basis of transformation of PVL, an oral premalignant lesion: An exome genome sequence study. **Source**: Genome 2 Life Research Grant 2017, University of Liverpool - £7,300. **Investigators**: Risk J, Okoturo E.
2. **Title**: Whole genome analysis of the premalignant lesion, PVL: comparison with oral squamous cell carcinoma. **Source**: Technology Directorate Voucher Scheme 2018, University of Liverpool - £7,500 (Matching fund). **Investigators**: Risk J, Okoturo E.
3. **Title**: Molecular Transformation of pre-malignant HNSCC lesion using High Throughput Sequencing-based Approach. **Source**: TETFUND research (PhD) grant 2016 - $ 150,000. **Investigators** - Okoturo E (PI) / Cancer Research Center, University of Liverpool.