BAOMS Research Grant – Interim Report no.2 - March 2020

Mr Karl F. B. Payne

Date awarded - March 2018. Grant received – October 2018 - £9,813

One year extension until October 2020

Original project title:

Circulating tumour DNA as a liquid biopsy and biomarker in head and neck squamous cell carcinoma (HNSCC)

Supervisors: Professor Hisham Mehanna and Mr Paul Nankivell

Institution – University of Birmingham

Background:

Despite advances in treatment, survival in HNSCC remains static and the incidence of recurrence/metastasis (R/M) is high. Tumour biopsies can rarely be taken from such R/M patients and treatment selection is therefore empirical, with consequent low rates of response. Furthermore, because HNSCC has significantly high intra-tumour genomic heterogeneity, any such samples cannot capture the entire mutational landscape of the tumour - thereby limiting their usefulness and leaving the clinician blind as to the emergence of clonal mutations driving resistance. A liquid biopsy utilises circulating fragments of tumour DNA (ctDNA) or tumour cells (CTCs) to assess tumour specific genomic alterations. This novel technique promises to deliver a non-invasive method to detect tumour specific proteogenomic heterogeneity in a dynamic fashion with the potential to provide biomarkers to guide targeted therapy.

Original research question:

Can ctDNA be used as a liquid biopsy in HNSCC to assess tumour heterogeneity and a biomarker to detect tumour recurrence?

Secondary research question:

Can circulating tumour cells be successfully isolated and characterised to assess intra-tumoural proteogenomic heterogeneity in HNSCC

Intiial efforts to answer the original research question using ctDNA have been slow - due to technical issues with ctDNA extraction and poor data from subsequent genomic sequencing. Success was achieved with methylation array sequencing in a small patient cohort (please see previous interim report for description of ctDNA methodology and results). Therefore, a decision was made to focus on an alternative liquid biopsy compartment – circulating tumour cells (CTCs), and to use funds from the research grant to optimise a novel microfluidic method of CTC enrichemnt and characterisation in HNSCC (the Parsortix platform).

Results:

- The Parsortix device has been successfully optimised using the FaDu and SCC040 HNSCC cell lines. By spiking HNSCC cells into donor blood at a concentration of 50-100 cells/ml (comparable to CTC count results from patient samples) we achieved a mean capture rate of 59% (n=10).
- Subsequently an antibody panel was optimised to detect CTCs in blood samples from HNSCC patients – to identify epithelial CTCs and those CTCs having undergoen and epithleial-to-mesenchymal transition (indicative of poorer prognosis).
- We demonstrated proof-of-principle in a small cohort of 4 patients – being able to detect and quantify sub-groups of CTCs using flow cytometry (data submitted to BJOMS).
- The above findings represent the first utilisation of the Parsortix device in HNSCC and are a promising foundation for future research in this area.

Presentation/publication of research output:

- Oral presentation at the BAOMS 2019 scientific meeting – ***"High-throughput methylation profiling of cell-free plasma DNA in head and neck cancer: a pilot study"***

- Mauscript submitted to the *British Journal of Oral and Maxillofacial Surgery* – *"**Validating the Parsortix™ system for enrichment and characterisation of circulating tumour cells in head and neck squamous cell carcinoma: a standardised approach to microfluidic circulating tumour cell biomarker discovery**"*
- Abstract submitted for BAOMS 2020 scientific meeting – ***"Microfluidic based circulating tumour cell isolation using the Parsortix platform in head and neck squamous cell carcinoma"***

Future work:

- Our aim is to achieve single cell proteogenomic characterisation of CTCs in HNSCC.
- RNAseq and mass cytometry will be used to characterise CTCs from a large patient cohort (50) and comparison made to the primary tumour and clinical outcomes such as treatment response.
- The above work will be funded by a CRUK Clinical PhD Fellowship.