**Annual report for the Pathological Society Small Grant Award**

**Title:** Characterising the DNA methylome in patients with metastatic melanoma treated with immune checkpoint blockade

**Name & Address:** Rosalin Cooper, Fairfax Laboratory, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford. Email: [rosalin.cooper@oncology.ox.ac.uk](mailto:rosalin.cooper@oncology.ox.ac.uk).

**Background and aims:**

Patients with metastatic melanoma (MM) are offered treatment with immune checkpoint blockade (ICB). Whilst some patients experience a durable response, many do not, and these drugs are associated with significant auto-immune toxicity (immune related adverse events - irAEs)1. Predictive markers of treatment response in this patient cohort would enable better patient stratification and prompt identification of non-responders, permitting earlier use of alternative treatment options.

Epigenetic modifications such as 5-methylcytosine (5mC) have been shown to have utility as diagnostic2,3 and predictive biomarkers in cancer4. Circulating cell-free DNA (cfDNA) is predominantly derived from circulating immune populations5, but may be tumour-derived in patients with cancer6. Assessment of epigenetic cfDNA modifications provides opportunity for biopsy-free assessment of both immune and tumour-based responses, however to date characterisation of 5mC cfDNA profiles in MM and in the context of ICB is limited.

The aims of this study were to characterise on-treatment cfDNA 5mC profiles in patients with MM, and explore their utility as potential diagnostic and predictive biomarkers.

**Results**:

Cell-free methylated DNA immunoprecipitation (cfMeDIP) was used to sequence 5mC-enriched cfDNA libraries across 24 patients both before and after ICB treatment and eight healthy donors. 5mC enriched regions were identified using the *MACS2*7 package and differential enrichment analysis was performed with *DiffBind*8.

Distinct 5mC cfDNA profiles were identified in MM patients versus healthy donors. Pairwise analysis following ICB identified 1298 differentially methylated regions (FDR<0.05). Pathway enrichment analysis revealed 5mC enrichment of genes associated with transcription and cell proliferation pathways and depletion across genes involved in GTPase signalling, oxidative stress and membrane organisation pathways. Notably, 5mC cfDNA profiles at baseline were associated with clinical outcome including disease progression by six months and irAEs.

**Conclusions:**

cfDNA 5mC profiles in MM patients are distinct from those in healthy individuals, and are modulated by ICB. 5mC cfDNA profiles are likely to represent both immune and tumour-based responses. These novel observations demonstrate that 5mC may have diagnostic and predictive utility in patients with MM treated with ICB. Further characterisation across our larger patient cohort (n>180 patients) would be valuable, and integration with existing RNA sequencing data to provide a ‘multi-omics’ characterisation of peripheral ICB responses is ongoing.

**How Closely Have the Original Aims been Met:**

Funding from the Pathological Society Small Grant scheme has allowed us to satisfy the aims of this proof-of-concept pilot study, which has successfully characterised 5mC cfDNA profiles in a MM cohort in the context of ICB, demonstrating diagnostic and predictive clinical utility.

**Outputs (including meeting abstracts, oral presentations, original papers, review articles) from the study in which the Pathological Society has been acknowledged:**

I will be presenting this work as a Poster Presentation at the Pathological Society/BDIAP Manchester Pathology meeting in July 2021. I also intend to publish these novel findings in a peer reviewed journal.

**References**

1. Ye, W. *et al.* Checkpoint-blocker-induced autoimmunity is associated with favourable outcome in metastatic melanoma and distinct T-cell expression profiles. *Br. J. Cancer* 1–9 (2021) doi:10.1038/s41416-021-01310-3.

2. Shen, S. Y. *et al.* Sensitive tumour detection and classification using plasma cell-free DNA methylomes. *Nature* vol. 563 579–583 (2018).

3. Nassiri, F. *et al.* Detection and discrimination of intracranial tumors using plasma cell-free DNA methylomes. *Nat. Med.* **26**, 1044–1047 (2020).

4. Jung, H. *et al.* DNA methylation loss promotes immune evasion of tumours with high mutation and copy number load. *Nat. Commun.* **10**, (2019).

5. Moss, J. *et al.* Comprehensive human cell-type methylation atlas reveals origins of circulating cell-free DNA in health and disease. *Nat. Commun.* **9**, 1–12 (2018).

6. Ma, X. *et al.* Cell-free DNA provides a good representation of the tumor genome despite its biased fragmentation patterns. *PLoS One* **12**, (2017).

7. Peak calling with MACS2 | Introduction to ChIP-Seq using high-performance computing. https://hbctraining.github.io/Intro-to-ChIPseq/lessons/05\_peak\_calling\_macs.html.

8. Stark, R. & Brown, G. *DiffBind: Differential binding analysis of ChIP-Seq peak data*.