

## MULTISPECTRAL IMMUNOHISTOCHEMISTRY REVEALS AN IMMUNOSUPPRESSIVE TUMOUR MICROENVIRONMENT IN ESOPHAGEAL ADENOCARCINOMA

<sup>1</sup>Charles Rayner\*, <sup>1</sup>Tyler Wooldridge, <sup>1</sup>Sunny Sunshine, <sup>2</sup>Izhar Bagwan\*, <sup>2</sup>Shaun Preston, <sup>1</sup>Hardev Pandha, <sup>2</sup>Nima Abbassi-Ghadi, <sup>1</sup>Nicola Annels. <sup>1</sup>University of Surrey, Guildford, UK; <sup>2</sup>Royal Surrey NHS Foundation Trust, Guildford, UK

**Background** Esophageal cancer – of which adenocarcinoma (EAC) is the predominant type in the UK – is a highly aggressive malignancy, with 5-year survival rates of approximately 15%.<sup>1</sup> Surgically resectable disease is treated with neo-adjuvant chemo+/-radiotherapy. The recent publication of Checkmate-577 supports the use of anti-PD-1 immunotherapy in EAC post chemoradiotherapy and surgery.<sup>2</sup> Beyond CD8<sup>+</sup> T cells and PD-1/PD-L1 expression, the immune tumour microenvironment of EAC is poorly understood. Further research is required to understand the immune phenotype of the EAC microenvironment and how this could be modulated to improve outcomes.

**Methods** Treatment naïve patients who had undergone esophago-gastrectomy at the Royal Surrey County Hospital, Guildford, United Kingdom, for esophageal adenocarcinoma, between April 2009 and March 2020 were identified through analysis of a retrospectively collected database. Formalin fixed paraffin embedded (FFPE) tissue was retrieved for multispectral immunohistochemical analysis. Adjacent normal and tumour tissues were stained with primary antibodies for CD68, CD57, CD8, CD4, FoxP3, Granzyme B, PDL1 and pancytokeratin. Cell populations and spatial relationships were analysed using the Phenoimager HT (Akoya Biosciences) and QuPath.<sup>3</sup>

**Results** A total of 44 patients were identified. Significantly increased populations in tumours compared to normal tissue were seen for CD68<sup>+</sup> (p<0.0001), FoxP3<sup>+</sup> (p=0.0001) and PDL1<sup>+</sup> (p<0.0001) cells. Geographical distribution analysis of these cells showed that the increases seen were mainly in the stromal compartment of the invasive margin of the tumour rather than the tumour core. Spatial relationship analysis demonstrated a survival benefit for those that had an increased median distance from tumour cells (PanCK<sup>+</sup> (p=0.002)) CD8<sup>+</sup> (p=0.0469) or CD4<sup>+</sup> (p<0.0001) cells to CD68<sup>+</sup> cells.

**Conclusions** Our results demonstrate that the immune tumour microenvironment of EAC appears to be an immunosuppressive phenotype. Increases in cell types associated with a pro-tumour microenvironment but no significant alteration in cytotoxic cell phenotypes (CD8<sup>+</sup>, CD57<sup>+</sup>, Granzyme B<sup>+</sup>) suggests that in order to improve response rates in EAC, immunotherapeutic strategies need to focus on decreasing immunosuppressive cells, as well as recruiting cytotoxic cells into the tumour microenvironment. Further works needs to be undertaken to fully understand the phenotype of macrophages seen in the TME of EAC.

### REFERENCES

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Ethics Approval Formalin fixed paraffin embedded (FFPE) tissue was retrieved for immunohistochemical analysis with ethical approval (ethical reference 19/NS/0196, IRAS 270878).

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