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AN HOLISTIC AND INTERGRATED APPROACH FOR INVESTIGATING THE BACTERIAL MICROBIOME, GENE EXPRESSION PROFILE AND IMMUNE CELL PROFILE IN THE NON-MUSCLE INVASIVE BLADDER CANCER TUMOUR MICROENVIRONMENT

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Background Bladder cancer (BC) is the 10th most common cancer world-wide with an estimated 570,000+ people being diagnosed in 2020.¹ It has been shown that communities of bacteria (bacterial microbiome) exist in both normal and cancerous tissues and can impact treatment efficacy (e.g. metabolising chemotherapeutics).²⁻³ We investigated the microbiome in formalin fixed paraffin embedded (FFPE) tumour tissue across different stages of BC compared to adjacent normal tissue to investigate differentially expressed (DE) bacteria. We also investigated DE bacteria between Bacillus Calmette Guerin (BCG) treatment responders vs non responders. These findings were then correlated to differential host immune gene expression and infiltrating immune cell profiles and spatial relationships within the tumour microenvironment (TME).

Methods Bacterial signatures within urine (n=56) and FFPE tissues (n=66), (matching patients n=44), derived from the Royal Surrey Country Hospital, Guildford, UK, were determined using 16s rRNA sequencing (V3-V4). Sequencing data were processed through QIIME2 and clustered into operational taxonomic units (OTUs). Alpha (Shannon and observed) and Beta (Bray-Curtis, weighted and unweighted uniFrac) diversity analysis was performed, with non-parametric statistics for determining significance. RNA extracted from FFPE BC tissues generated gene expression data using the Nanostring IO360 panel (a 770 gene CodeSet) and was correlated to tumour microbiome profiles using SparCC analysis. 9 colour multiplex immunohistochemistry (mIHC) using Phenoimager HT (Akoya Biosciences) was also performed to investigate and spatially define immune cell types (CD4, CD8, CD68, CD57, FOXP3, GRZB, PD-L1, PANCK, DAPI), within tumour tissues (n=71).

Results BC bacterial microbiome showed decreased diversity and composition with disease progression (P<0.01). The bacterial profiles between urine and FFPE cancer tissues revealed independent groups (P<0.01), showing urine is not an accurate proxy. The bacterial microbiome of cancerous and normal tissue, and between responders vs non responders shared mostly similar bacteria, though differentially expressed bacteria were found. Immune cell profiles and spatial relationships determined via mIHC, showed differences between stromal and tumour regions, and low grade (LG) and high grade (HG) disease. Analysis has also shown links between bacteria, immune gene expression and immune cells populations.

Conclusions The analysis of the microbiome in BC has clearly shown differentially expressed bacteria going from low grade to high grade disease. Further investigation of bacteria influencing immune cell phenotype is currently being performed. The potential influence these bacteria have on the tumour immune microenvironment and thus disease and treatment outcomes will provide the rationale for pursuing approaches to modulate the tumour microbiome for improved therapeutic outcomes.

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Ethics Approval Patient derived Formalin fixed paraffin embedded (FFPE) tissue and urine samples for analysis were received with ethical approval (ethical reference 12/LO/1661).

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