THE TUMOR MICROBIOME CORRELATES WITH RESPONSE TO IMMUNE CHECKPOINT INHIBITORS IN RENAL CELL CARCINOMA

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Background Immune checkpoint inhibitor therapy, or ICI, is currently the most successful treatment option for patients with renal cell carcinoma (RCC). However, only 20% of patients have a durable response,¹ driving a significant need to improve treatment outcomes. The tumor microbiome has recently been shown to play a role in chemotherapy-based treatment outcomes, but, to our knowledge, no study has explored its role in response to ICIs.²⁻⁴

Methods Tumor samples were collected from 22 patients with RCC as a part of the Total Cancer Care program at The Ohio State University Comprehensive Cancer Center. Raw RNA-seq reads from these biopsies, as well as data on the responses to ICI therapy were collected. Response evaluation was based on RECIST v1.1 criteria with complete or partial response, or stable disease classified as "Responders," and progressive disease classified as "Non-responders". The RNA-seq reads were processed through a pipeline developed by the Spakowicz lab, known as ExoTIC (Exogenous sequences in Tumor and Immune Cells), to carefully identify exogenous sequences.^{5,6} Reads that don't align to the human reference genome are meticulously filtered of (1) common laboratory contaminants, (2) taxa that inversely correlate with input RNA quantity, and (3) taxa commonly found in the negative controls of microbiome experiments. DESeq2 was used to perform a differential abundance analysis on the comparison groups at every taxonomic level.

Results The 22 patients with RCC range from 22 to 74 years of age at diagnosis, are 72.7% male, and 54.5% responded to ICIs. Exogenous taxa are identified in the tumor RNAseq, including bacteria, fungi, and viruses (figure 1). Within the tumors responsive to immunotherapy, there was found to be a significant enrichment of certain microbial species, including *Bacillus thuringiensis*, *Comamonas testosteroni*, *Colletotrichum higginsianum*, and *Elaeis guineensis*. Comparatively, the cohort of non-responsive tumors was found to have a significant enrichment of *Candidatus Promineofilum breve*, *Clostridioides difficile*, *Nocardia cyriacigeorgica*, *Streptomyces* sp. CdTB01, and *Streptomyces venezuelae* (figure 2).

Conclusions We found that prior to ICI treatment the tumor microbiome of patients with RCC whose tumors responded to immunotherapy vary from those that did not respond to treatment. This implies that a therapeutic target to modify the tumor microbiome to improve treatment outcomes. Future research will evaluate whether these correlations are causally associated with outcomes and will evaluate their effect on the tumor microenvironment including immune cell infiltration.

Abstract 942 Figure 1 Relative abundances of exogenous taxa found in tumor RNAseq are shown in a stacked bar plot

Abstract 942 Figure 2 Differential abundance analysis of taxa found within tumor RNAseq data by the exotic pipeline. Colored points represent significantly (pvalue < 0.05) enriched taxa with a high (>2.5) fold-difference in abundance between the groups

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Ethics Approval Data were obtained through an IRB-approved Honest Broker protocol (2015H0185) supporting the Total Cancer Care protocol 2013H0199.

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