Background
Emerging evidence supports an important role for the tumor microbiome in relation to oncogenesis, cancer immune phenotype, cancer progression and treatment outcomes in a number of malignancies. In this study, we investigated the metastatic melanoma tumor microbiome and potential roles in association with clinical outcomes, such as survival, in patients with metastatic disease treated with immune checkpoint inhibitors (ICIs)\(^1\)\(^-\)\(^^3\).

Methods
Baseline tumor samples were collected from 71 patients with metastatic melanoma prior to treatment with ICIs. Bulk RNA-seq was conducted on the FFPE tumor samples. Clinical outcome following ICI treatment was evaluated as overall survival (> 24 versus < 24 months). The RNA-seq reads were processed to carefully identify exogenous sequences using ExoTIC (Exogenous sequences in Tumor and Immune Cells)\(^4\)\(^,\)\(^5\). Reads that did not align to the human reference genome were 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 71 patients with metastatic melanoma ranged in age from 24 to 83 years, 55% were male, and 55% survived > 24 months following the initiation of ICI treatment. Exogenous taxa were identified in the tumor RNAseq, including Fusobacterium nucleatum, Porphyromonas asaccharolytica, Nocardia m Kang, and Mollivirus sibericum. Comparatively, the cohort of non-responsive tumors (< 24 months survival) was found to have a significant intra-tumor enrichment of Fungi, as well as the bacteria Delftia lacustris, Enterobacter hormaechei, Psedomonas fluorescens, and Moraxella osloensis (figure 2).

Conclusions
In investigating the melanoma tumor microbiome utilizing baseline tumors (prior to initiating ICI) we found significant variations in the exogenous taxa associated with patient outcomes following ICI treatment. Our findings warrant further investigations and potentially support therapeutic strategies to modify the tumor microbiome in order to improve treatment outcomes with ICIs. Ongoing research is evaluating whether these correlations are causally associated with outcomes and evaluating their effect on the tumor immune microenvironment and immune cell infiltration.

Abstract 1311 Figure 1
A stacked bar plot showing the relative abundances of exogenous taxa found in tumor RNAseq. Taxa are shown on the phylum level and are ordered by the relative abundance of <i>Uroviricota</i>.

Abstract 1311 Figure 2
Differential abundance analysis of taxa found within tumor RNAseq data by the exotic pipeline. Colored points represent significantly (p-value < 0.05) enriched taxa with a high (>1.00) fold-difference in abundance between the groups.

References