**Background** Mucosal associated invariant T (MAIT) cells are innate like T-cells that recognize non-peptide metabolite antigens presented on monomorphic MHC related-1 (MR1) making them an attractive off the shelf tool for cancer immunotherapy. In this study, we investigate the function of MAITs in the tumor microenvironment of neoadjuvant anti-PD1 treated lung cancer patients (NCT02259621).

**Methods** Paired single cell RNA and TCR sequencing data was mined. TCR capture techniques were used for functional antigen recognition assay. 16S RNA and Whole genome sequencing was performed on patient tumor lung and paired normal lung for microbiome sequencing.

**Results** Paired single cell RNA/TCR sequencing analysis revealed an oligoclonal expansion of MAITs comprising of canonical TRAV1–2+ and non-canonical MAITs TRAV1–2-. TCR capture from canonical MAIT clonotypes confirmed their *in vitro* recognition of 5-OP-RU, bacteria derived riboflavin derivative. However, the nature of antigens recognized by non-canonical MAITs remains poorly understood. On mining transcriptional profiles of MAITs, we observed that canonical MAITs were characterized by high clonal amplification whereas the non-canonical clonotypes expressed a strong activation/exhaustion gene signature. This suggests that both these MAIT populations recognize different types of antigens, differentially activate, and respond to immune checkpoint blockade (ICB), the non-canonical MAITs being highly dysfunctional. Using bacterial 16S RNA amplicon and metagenomics sequencing, we seek to identify microbial and metabolomics signatures associated with MAIT activation and response to ICB.

**Conclusions** We found that conventional MAITs do not express neoantigen specific conventional T-cell signature. Our findings will provide further insights into the nature of ligands recognized by MAITs and the mechanisms associated with MR1 dependent recognition and tumor killing. Investigating the role of unconventional T-cells in response to ICB has the potential to reveal new tumor-associated antigens which could open avenues into innovative combination immunotherapies.