Background The gut microbiome is a known modulator of response to checkpoint inhibitors.1-4 MRx0518 is a strain of Enterococcus gallinarum that was isolated from a healthy human fecal sample. Administration of MRx0518 in pre-clinical cancer models results in anti-tumor effects and immune system modifications potentially contributing to therapeutic effects of checkpoint inhibitors. We hypothesized that a PD-1 checkpoint inhibitor in combination with MRx0518 would decrease suppressive myeloid cells and increase T-cell activation.

Methods

Study design: Patients who had developed resistance to checkpoint inhibitors received MRx0518 (1 x 10^{10} to 1 x 10^{11} CFU) PO BID and 200mg pembrolizumab IV Q3W for up to 2 years or disease progression. Responders are defined as patients achieving clinical benefit (CR, PR or SD ≥ 6months per RECIST v1.1).

Flow cytometric analysis: PBMCs from baseline (BL) and cycle 4 day 1 (C4D1) were subjected to immune profiling. Normal donor (ND, n=9) PBMCs serve as controls for non-responder (NR, n=33) and responder (R, n=11) BL samples.

Circulating biomarker assay: Cytokines were assessed in plasma collected at BL (n=27) and C4D1 (n=27) using a kit from Meso Scale Discovery.

Statistical tests: Non-parametric ANOVA and Mann-Whitney test or Wilcoxon matched-pairs signed rank test were utilized for flow cytometry data and paired T-test for cytokine analysis.

Results

At BL, expression of HLA-DR on mDC is reduced and the frequency of HLA-DR negative monocytes is increased in patients (p<0.05) suggesting a higher degree of suppressive myeloid cells prior to combination therapy. Expression of PD-L1 and PD-L2 on mDC and monocytes is higher in patients at BL (p<0.05). Checkpoint receptor expression and activation markers on T cells (both CD4+ and CD8+) is higher in patients at BL, including CTLA4 (p<0.01), PD-1 (p<0.05), Tim3 (p<0.05), OX40 (p<0.001) and Ki67 (p<0.05). CTLA4, PD-1, and Tim3 (p<0.05) expression on NK cells are higher in patients at BL. Overall, the circulating immune microenvironment is immuno-suppressed in patients at BL irrespective of subsequent clinical outcome.

Upon treatment, HLA-DR+ myeloid cells are increased, PD-L1 expression on HLA-DR+ myeloid cells is consistently reduced, and the frequency of CD8+ T cells is increased in R patients (p<0.05). IL-6 and MIP-1α are increased in circulation in NR upon treatment (p<0.05).

Conclusions Immune activation was recovered in R patients with MRx0518 and anti-PD-1 combination therapy. Immune changes associated with improved outcome include: 1) increased expression of HLA-DR and decreased PD-L1 expression on myeloid cells and 2) increased CD8+ T-cell frequencies in circulation.