

## COMBINATION MRX0518 AND ANTI-PD-1 OVERCOMES CHECKPOINT INHIBITOR RESISTANCE VIA MYELOID MODULATION

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**Background** The gut microbiome is a known modulator of response to checkpoint inhibitors.<sup>1-4</sup> 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<sup>10</sup> to 1 x 10<sup>11</sup> 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.

**Acknowledgements** Study is funded through the 4D Pharma strategic alliance with MD Anderson Cancer Center. We appreciate the support of all the patients and their families for their participation in the study.

**Trial Registration** ClinicalTrials.gov Identifier: NCT03637803

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**Ethics Approval** This study was written and conducted in accordance with the principles from the Declaration of Helsinki. Written informed consent was provided by all study participants or their legal representatives. The study was approved by the University of Texas MD Anderson Cancer Center's Institutional Review Board.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0838>