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 [1-4]
- Response to checkpoint inhibitors is associated with higher microbiota diversity
- MRx0518, from 4D Pharma, is a strain of *Enterococcus gallinarum* that was isolated from a healthy human gut
- Administration of MRx0518 in pre-clinical cancer models significantly reduced tumor growth and promoted anti-tumoral response by increasing cytokines and T-cells in the TME [5]

Hypothesis

- PD-1/PD-L1 checkpoint inhibitors in combination MRx0518 will result in decreased frequency of suppressive myeloid cells and increase in T-cell activation

Methods

Study design:

- Patients who had developed resistance to checkpoint inhibitors received MRx0518 and pembrolizumab for up to 2 years or disease progression.
- Responders are defined as patients achieving clinical benefit (CR, PR or SD ≥ 6 months 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) a 40-plex assay from Meso Scale Discovery.

Statistical test

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. P value: 0.01 ≤ * <0.05, 0.001 ≤ ** <0.01, 0.0001 ≤ *** <0.001, **** <0.0001

Abbreviations

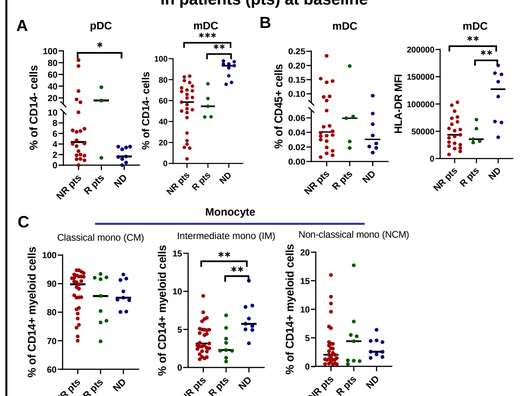
- Non-R = non responder (SD < 6M, PD or iSD)
- R = responder (SD ≥ 6M, PR or CR)
- ND = normal donor

References

1) Gopalakrishnan V et al. *Cancer Cell* 2018; 33:570-580
2) Routy B et al. *Science* 2018; 359: 91-97
3) Baruch F et.al. *Science* 2021; 371: 602-609.
4) Davar D et al. *Science* 2021; 371: 595-602.
5) Couturier-Maillard A et al. Poster (#6201)_AACR 2020.

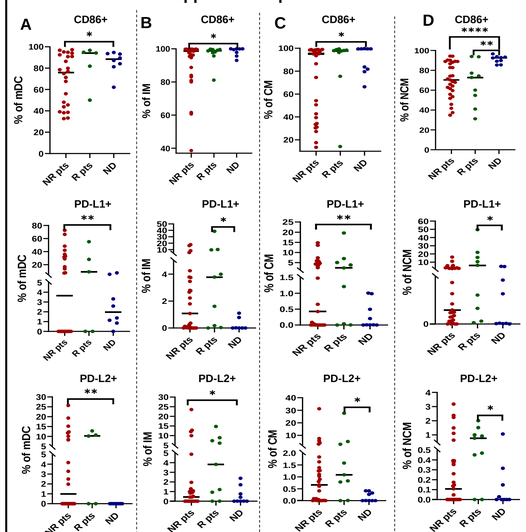
Results

Fig 1. High degree of suppressive myeloid cells in circulation in patients (pts) at baseline



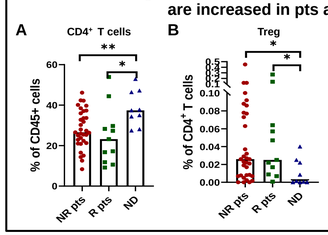
A. Flow cytometric analysis of pDC and mDC within the CD14- cell population in NR , R and ND PBMC respectively. **B.** Flow cytometric analysis of mDC within the CD45⁺ cell population, and HLA-DR MFI on mDC, in NR, R and ND PBMC respectively. **C.** Flow cytometric analysis of three subsets of monocytes in NR, R and ND PBMC. Mann-Whitney test was used for statistical analysis.

Fig 2. Circulating immune microenvironment is immuno-suppressed in pts at baseline



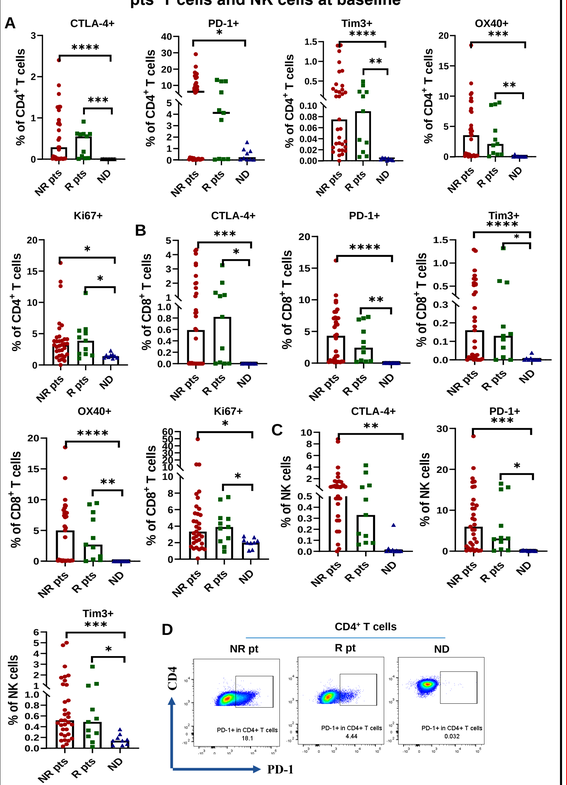
Flow cytometric analysis of CD86, PD-L1 and PD-L2 expression within mDC (**A**), and three subsets of monocytes, including IM (**B**), CM (**C**) and NCM (**D**) in NR, R and ND PBMC. Mann-Whitney test was used for statistical analysis.

Fig 3. CD4⁺ T cells are reduced and Tregs are increased in pts at baseline



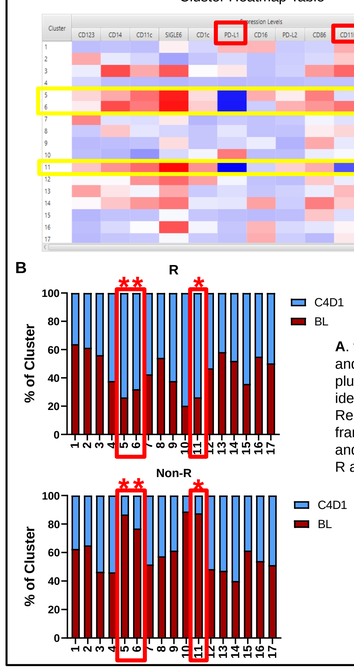
Flow cytometric analysis of CD4⁺ T cell frequency within the total CD45⁺ population (**A**), as well as Treg (CD4⁺CD25⁺FoxP3⁺) frequencies within the CD4⁺ T cell population (**B**). Mann-Whitney test was used for statistical analysis.

Fig 4. Higher expression of checkpoint receptors on pts' T cells and NK cells at baseline



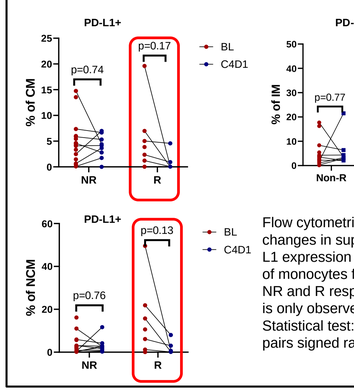
Flow cytometric analysis of inhibitory and activation markers on CD4⁺ T cells (**A**), CD8⁺ T cells (**B**) and NK cells (**C**) respectively, in NR, R and ND. **D**. Representative flow data comparing PD-1 expression on CD4⁺ T cells in NR, R and ND. Mann-Whitney test was used for statistic analysis

Fig 5. HLA-DR⁺, CD86⁺ and PD-L1⁻ myeloid cells are increased in R pts upon treatment



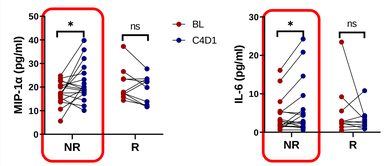
A. tSNE, UMAP, X-shift and cluster explorer plugins within Flowjo identified 17 clusters. **B.** Relative distribution of framed clusters in BL and C4D1 time point, in R and NR respectively.

Fig 6. PD-L1 expression on monocytes is reduced in R pts upon treatment



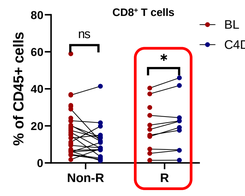
Flow cytometric analysis of dynamic changes in suppressive ligand PD-L1 expression across three subsets of monocytes from BL to C4D1, in NR and R respectively. Difference is only observed in R patients. Statistical test: Wilcoxon matched-pairs signed rank test.

Fig 7. MIP-1α (CCL3) and IL-6 are increased in NR pts in circulation upon treatment



Cytokines were assessed in plasma from pts. Dynamic changes from BL to C4D1 were assessed in NR and R respectively. A paired t-test was used for statistical analysis.

Fig 8. Circulating CD8⁺ T-cell frequencies increase in R pts upon treatment



Flow cytometric analysis of CD8⁺ T cells within the total CD45⁺ population, in NR and R respectively, at BL and C4D1. Statistical test: paired t-test.

Conclusions

1. The circulating immune microenvironment appears suppressive in patients at baseline:

- Low HLA-DR, high PD-L1 and high PD-L2 expression on myeloid lineages
- Higher expression of PD-1 and CTLA4 on T-cell subsets

2. Suppression of the circulating immune microenvironment is relieved upon treatment in R, but not in Non-R patients

3. Increased MIP-1α (CCL3) and IL-6 upon treatment may be associated with Non-R.

4. Early on-treatment (C4D1) immune changes are associated with improved patient outcome:

- Increased expression of HLA-DR
- Decreased PD-L1 expression
- Increased CD8⁺ T-cell frequencies

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