5945. Transcriptional analysis of TME in MC38 colon carcinoma model following checkpoint inhibition

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Introduction

- Colorectal cancer (CRC) is one of the most common cancers with 200,000 U.S. cases per year. Adenocarcinoma is the most common type of CRC, making up 95 percent of all CRC cases. Checkpoint inhibitors used for CRC have shown efficacy for a specific subset of patients.
- MC38 is one of the robust preclinical tumor models in immuno-oncology due to its characteristics as an immunologically warm tumor and response to some immunotherapies.
- To identify underlying factors that influence differences in treatment response, the tumor microenvironment (TME) of the MC38 model was analyzed following the treatment with anti-programmed cell death protein 1 (PD-1) and anti-cytotoxic T lymphocyte associated protein 4 (CTLA-4).
- Labcorp *In Vitro* Preclinical Oncology uses molecular signatures within the TME in preclinical models to help inform both test agent mechanism and combination strategies.
- MC38 syngeneic colon tumor model is used as an example of how phenotypic signatures can be leveraged for early identification of predictive biomarkers and for rational design of combination therapies with checkpoint inhibitors to improve patient outcome.

Methods

- MC38-NCI.TD1 cells were implanted subcutaneously into the axilla of female C57BL/6J mice. The dosing was administered once the tumors became established and tumor progression was monitored by caliper measurements. Anti-PD-1 (RMP1-14) or isotype control (rat IgG) and anti-CTLA-4 (9D9) or isotype control (mouse IgG) (Bio X Cell) were dosed 3 times post MC38 implant. All animal work was performed in an AAALAC-accredited facility, in alignment with applicable animal welfare regulations and with predetermined humane euthanasia criteria on all studies.
- **Flow cytometric analysis:** subcutaneous tumors were harvested, dissociated (Miltenyi Biotec), and labeled with fluorescent antibodies. Immune cell absolute counts were quantified using Precision Count Beads™ (BioLegend). The data was acquired on a Cytoflex® LX (Beckman Coulter) flow cytometer and analyzed using FlowJo® software (BD). The statistical analysis was performed using Mann-Whitney U tests (*p<0.05).
- **NanoString nCounter® analysis:** mRNA was extracted from paraffin embedded tumor samples and the expression was analyzed using the mouse PanCancer IO 360™ panel (NanoString Technologies). ROSALIND and nSolver™ advanced analysis platforms were used to measure differential expression. Global significance scores were calculated. Criteria for significant genes is ± 1.5-fold change with p-adj<0.05.

Figure 1. Growth kinetics of MC38 tumors following treatment with anti-PD-1 and anti-CTLA-4. MC38 established disease is minimally responsive to checkpoint blockage. Arrows indicate treatment days.

Results

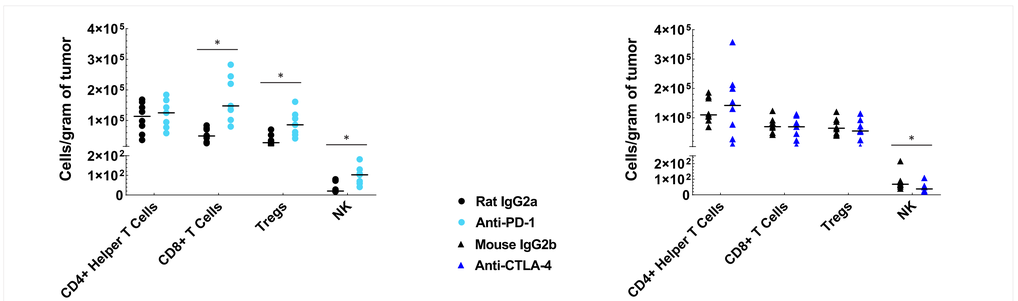


Figure 2. Immune subset infiltration of lymphoid subsets was measured by flow cytometry. PD-1 blockade increases lymphocyte infiltration into MC38 tumors.

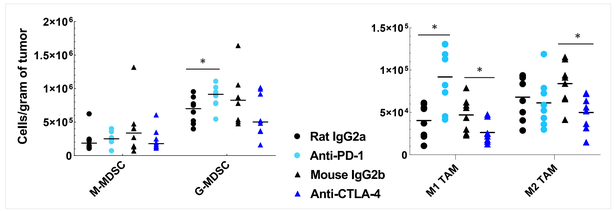


Figure 3. Immune subset infiltration of myeloid subsets was measured by flow cytometry. Checkpoint blockade results in changes in myeloid immune infiltration into MC38 tumors.

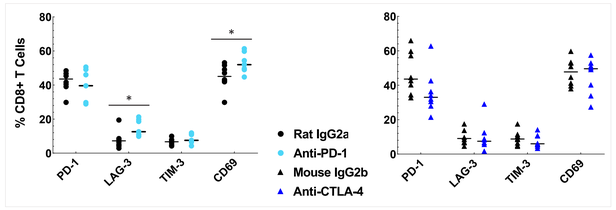


Figure 4. Flow cytometry measurement of functional markers on T cells. Checkpoint blockade triggers modest biomarker changes on CD8+ T cells in tumors.

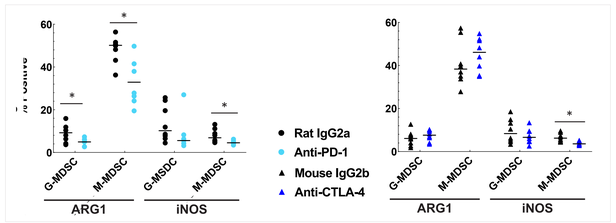
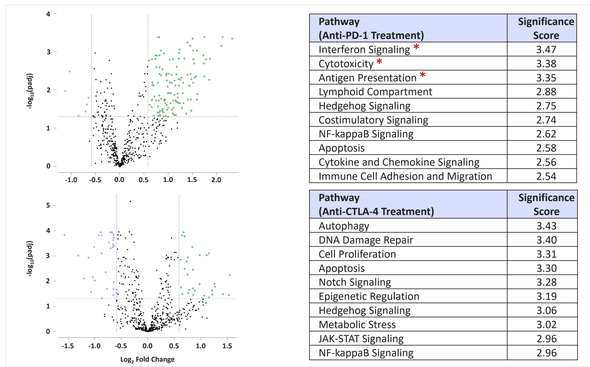


Figure 5. Flow cytometry measurement of functional markers on myeloid subsets. Myeloid biomarkers of immunosuppression are downregulated with PD-1 checkpoint blockade.



Pathway (Anti-PD-1 Treatment)	Significance Score
Interferon Signaling *	3.47
Cytotoxicity *	3.38
Antigen Presentation *	3.35
Lymphoid Compartment	2.88
Hedgehog Signaling	2.75
Costimulatory Signaling	2.74
NF-kappaB Signaling	2.62
Apoptosis	2.58
Cytokine and Chemokine Signaling	2.56
Immune Cell Adhesion and Migration	2.54

Pathway (Anti-CTLA-4 Treatment)	Significance Score
Autophagy	3.43
DNA Damage Repair	3.40
Cell Proliferation	3.31
Apoptosis	3.30
Notch Signaling	3.28
Epigenetic Regulation	3.19
Hedgehog Signaling	3.06
Metabolic Stress	3.02
JAK-STAT Signaling	2.96
NF-kappaB Signaling	2.96

Figure 6. PanCancer IO 360™ panel identified 131 genes that were differentially expressed in MC38 tumors following anti-PD-1 whereas 61 genes were modulated with anti-CTLA-4 treatment. (Left) Volcano plots representing differentially expressed transcripts in the treatment group compared to the isotype control group (green and purple data points). Vertical lines mark a fold change of ± 1.5. The horizontal line marks a p-adj cutoff at 0.05. (Right) Global significance scores were calculated using the ROSALIND platform and the 10 most significant modulated pathways are presented in the table. *Pathways examined further in Figure 8.

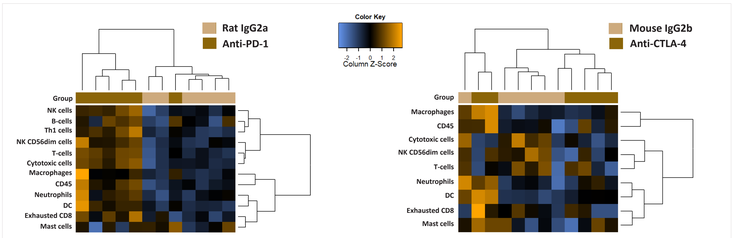


Figure 7. Immune cell abundance with anti-PD-1 (left) and anti-CTLA-4 (right) treatments. Immune cell gene expression signatures demonstrate activation phenotypes with anti-PD-1 treatment but not with anti-CTLA-4.

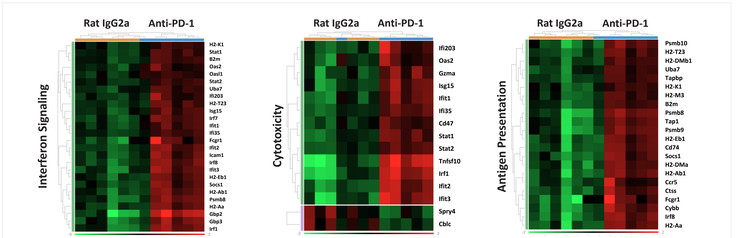


Figure 8. Heatmaps from anti-PD-1 treatment to compare differential gene expression within 3 selected gene sets. Tumors from mice (n=6/group) from anti-PD-1 and isotype control groups are shown. Only genes that were differentially expressed are listed.

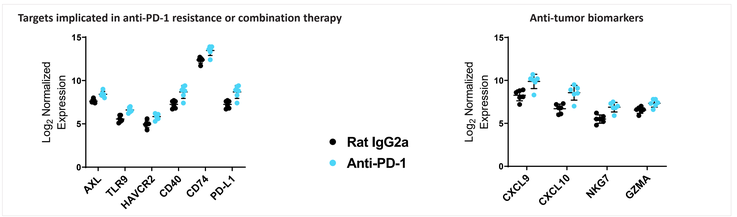


Figure 9. The 131 genes that were differentially expressed upon anti-PD-1 treatment included targets for both anti- and pro-tumor genes in MC38 tumors.

Conclusions

- The MC38 colon tumor model is minimally responsive to anti-PD-1 therapy and refractory to anti-CTLA-4 treatment under the conditions tested.
- Flow cytometry revealed immunological changes in the MC38 TME following anti-PD-1, but anti-CTLA-4 response was mostly static.
- Changes in gene expression demonstrated by nCounter® complement an immune activation phenotype with anti-PD-1 therapy despite minimal efficacy. This observation can allow for potential synergistic efficacy in combination with immuno-oncology drug candidates.
- The importance of using multiple modalities is essential for an in-depth understanding of the TME.

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