Characterization of a novel T-cell population associated with melanoma patient outcomes.

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Background

Immune checkpoint blockade (ICB), such as the use of antibodies against PD1/PDL1 or CTLA4, is a central therapeutic option for patients with advanced metastatic melanoma. While the key biological mechanisms surrounding ICB are well known, many aspects of immune or tumor cell interactions are not well understood. Glycoproteins such as CD38 and CD39 function as ectoenzymes with catalytic activity that can influence neighboring cells. In a previous study, we described a novel population of T-cells, CD4+CD38+CD39+CD127-GARP-, which are associated with nivolumab resistance[1].

Patient Characteristics

Checkmate 064 (CA209064)[2]
- Assess the potential synergistic activity of nivolumab and ipilimumab
- sequential combination regimens

Cohort A:
- Nivolumab followed by Ipilimumab

Cohort B:
- Ipilimumab followed by Nivolumab

Baseline Patient Specimens
- tumor infiltrating lymphocytes (TILs)
- Peripheral blood mononuclear cells (PBMCs)
- Collection at Week 0

Subject number	Cohort	TIL-PBMC pairs	Response
Pt_05	B	match	PR
Pt_09	B	match	PR
Pt_20	B	match	PD
Pt_22	B	match	PD
Pt_25	A	match	PD
Pt_11	A	TIL only	PR
Pt_27	A	match	PD
Pt_16	A	PBMC only	PR
Pt_19	A	TIL only	PR

Methods

CITE-seq[3] (Cellular Indexing of Transcriptome and Epitopes by sequencing)
- a multiplex protein detection system
- Single cell mRNA sequencing

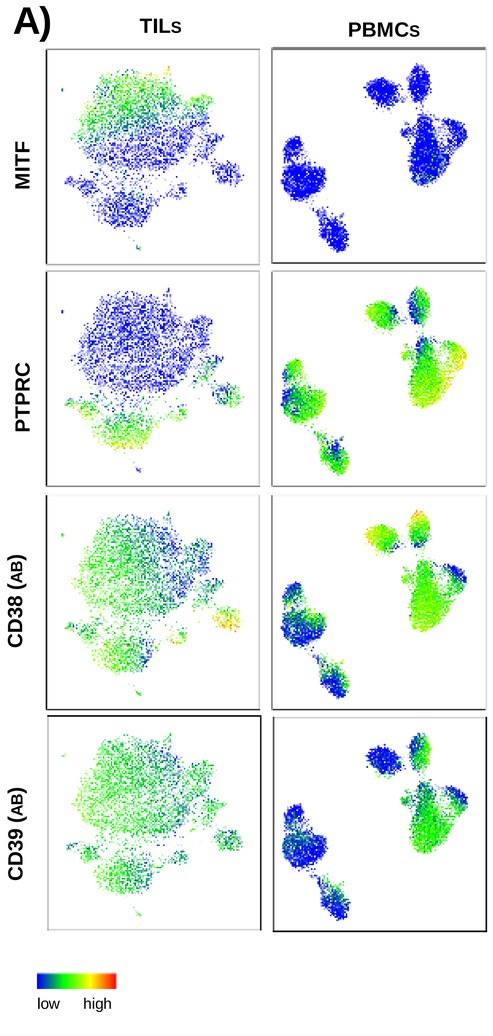
BD Bioscience Rhapsody System
- Single-Cell capture
- Oligo-tagged Antibodies panel
- Human mRNA Immune Response Panel a
- Custom Panel mRNA
- Illumina NovaSeq and MidSeq

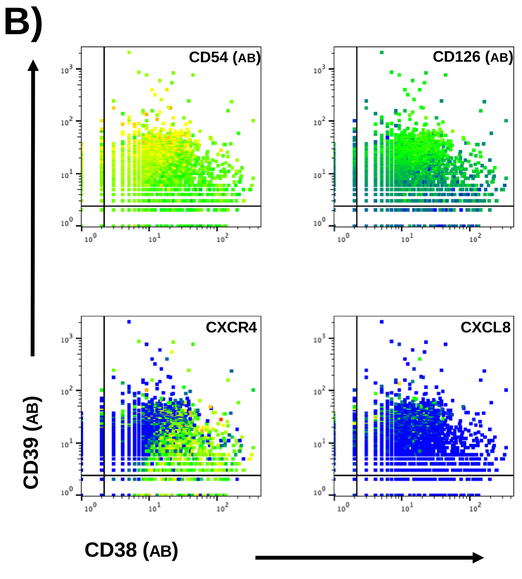
Antibodies and Gene Panel

Abbreviated Antibody Panel		
CD3	CD14	CD274
CD4	CD19	CD28
CD8	CD2	CD40
CD39	CD24	CD86
CD38	CD34	CD94
CD54	CD5	CD86
CD126	CD9	CD122
CD127	CD152	CD25

Abbreviated Gene Panel		
CCL1	IL6	CXCL2
CCL2	CCL3	CXCL3
CCR10	IL2RB	CXCL8
CCR7	GZMB	IFNGR1
MITF	CXCR1	TNF
PTPRC	CXCR2	IL32
IL1B	CXCR4	HLA-DMA
CXCL16	CXCR5	HLA-DRA

Single Cell Analysis

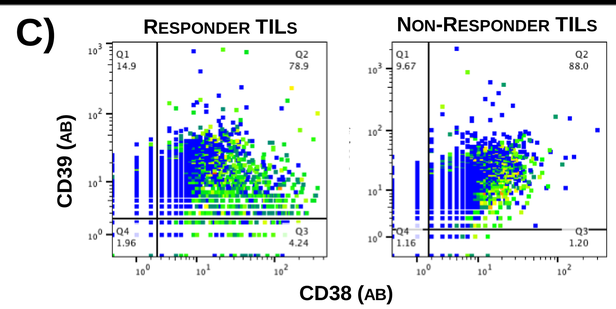


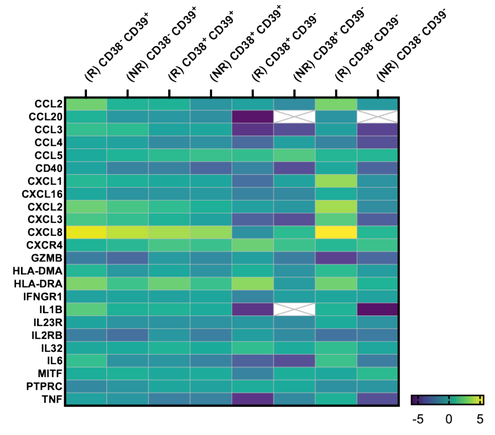


A) Dimensionality reduction for TILs and PBMCs: MITF is a transcription factor that regulates melanonocyte development. PTPRC: is part of the protein tyrosine phosphatase (PTP) family and is a common marker for immune cells. CD38 (cyclic ADP ribose hydrolase), can function as a receptor or an enzyme. CD39 (ectonucleoside triphosphate diphosphohydrolase-1) is a cell surface enzyme that hydrolyzes ATP and ADP.

B) CD38 and CD39 cell populations in TILs: TIL specimens were gated for CD38 and CD39 cell populations. Highlighted are protein expression for CD54 and CD126, and gene expression for CXCR4 and CXCL8.

Patient Responses





CD38 and CD39 cell populations: C) TILs from Patients responders and non-responders to ICB. Highlighted is PTPRC expression. **D)** Gene expression for CD38 and CD39 cells in Responders and Non-Responders.

Conclusions

- We were able to detect CD38+C39+ cells in patient TILs.
- Expression for CD54, a cell adhesion molecule, and CD126, the receptor for IL6 were increased in this cell population.
- Differential gene expression analysis suggests these cells signal through cytokines and chemokines such as TNF, IL1B, IL6, CXCL8, and CXCR4.
- Baseline frequency of CD38 and CD39 and gene expression patterns differ in TILs from patients responsive or non-responsive to ICB.

References and Support

1. Woods et al. *Nivolumab and ipilimumab are associated with distinct immune landscape changes and response-associated immunophenotypes.* **JCI Insight** 2020
2. Stoeckius et al. *Simultaneous epitope and transcriptome measurement in single cells.* **Nature Methods** 2017
3. Webber, et al *Sequential administration of nivolumab and ipilimumab with a planned switch in patients with advanced melanoma (CheckMate 064): an open-label, randomised, phase 2 trial.* **The Lancet Oncology** 2016

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