EVALUATION OF IMMUNOMODULATORY RECEPTOR/LIGAND EXPRESSION ON MATCHED HUMAN BIOSPECIMENS

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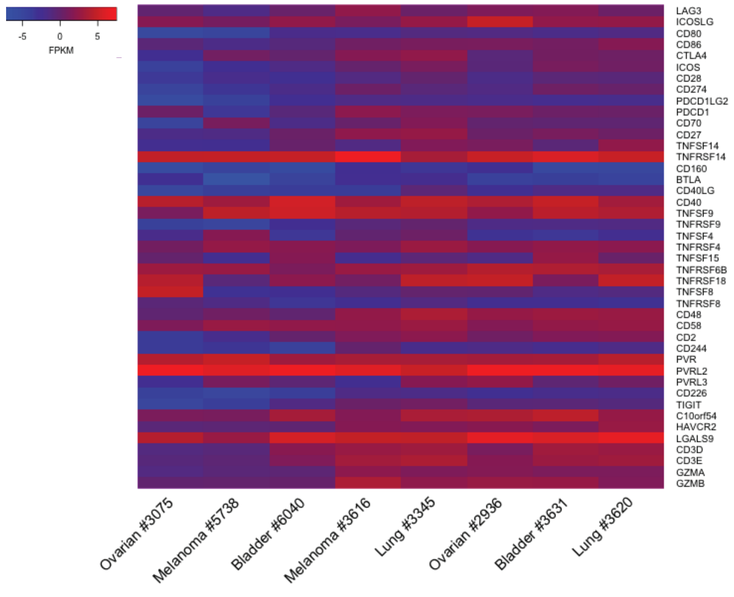
ABSTRACT

The integration of immunomodulatory receptor signaling is crucial for the activation status of responding T cells, and modulation of these receptors, and their ligands, may be of therapeutic benefit. Indeed, recent breakthroughs in checkpoint inhibitor therapies, and in particular those that target the PDL1/PD1 interaction, have demonstrated success in numerous oncological indications. Understanding the expression of these receptors and their cognate ligands within the complex cellular architecture of solid tumors will be fundamentally important to the design of the next-generation of immunotherapies.

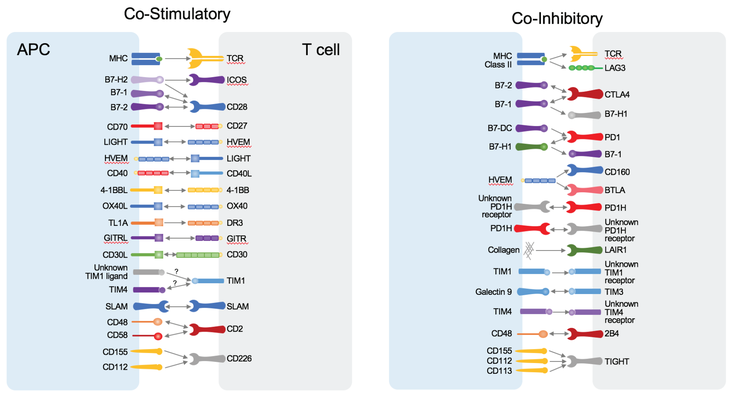
Bulk RNASeq analysis of primary human tumor tissue revealed the expression of numerous co-stimulatory (LIGHT/HVEM, 41BB/41BBL, OX40/OX40L, GITR/GITRL) and co-inhibitory (Lag3, VISTA, PVR/PVRL2/TIGIT, Tim3/Galectin-9) receptors and ligands within the tumor microenvironment. Using multiparametric flow cytometry, we have profiled the expression of these immunomodulatory receptors and their respective ligands on the major cellular components of the tumor microenvironment and correlated it with expression on cellular subsets within matched peripheral blood.

IMR Expression by Bulk RNAseq



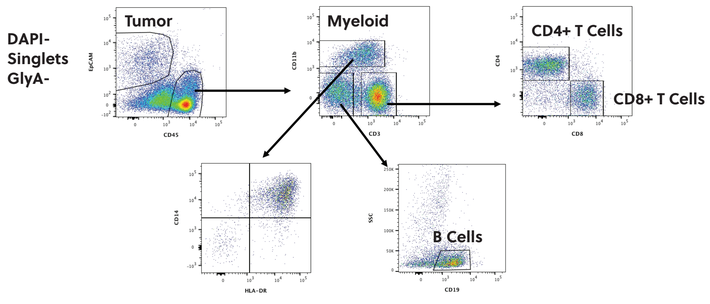
Bulk RNASeq analysis of dissociated tumor cells reveals dynamic immunomodulatory receptor expression. RNASeq was performed on 8 unique dissociated tumor samples (2 bladder cancer, 2 lung cancer, 2 melanoma, and 2 ovarian cancer patients). Expression of immunomodulatory receptors and their respective ligands was analyzed. T cell signature genes (Cd3e, Cd3d, Gzma, and Gzmb) were included to identify cold versus hot tumors. Data represent the log2 FPKM values for each sample.

IMR Receptor-Ligand Pairs



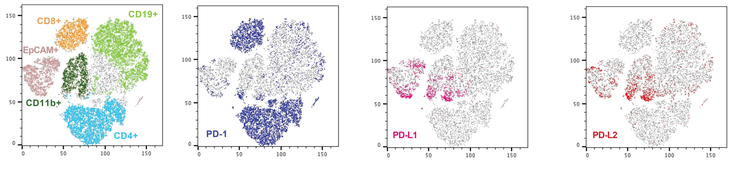
T cells have surface expression of a complex network of co-stimulatory and co-inhibitory receptors that modulate T cell responsiveness. The ligands for these receptors are expressed on numerous cellular populations, including antigen-presenting cells and tumor cells.

14 Color Flow Panel for IMR Evaluation



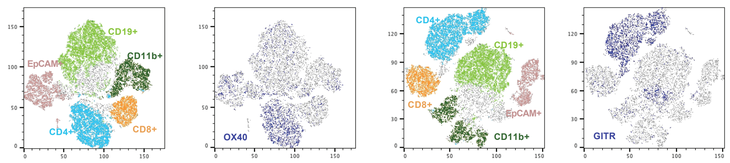
Dissociated tumor cells were analyzed by flow cytometry for 13 cell surface markers, as well as the live/dead discriminator DAPI, to identify the major tumor and immune cell populations present in the tumor microenvironment.

Inhibitory PD-1/PD-L1/PD-L2 Expression



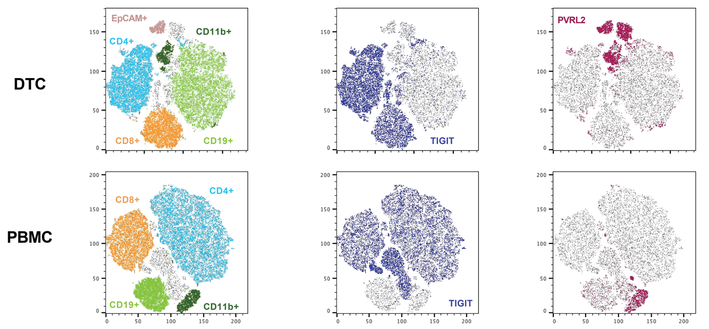
Dissociated tumor cells from a lung cancer patient were analyzed by flow cytometry for tumor cells (EpCAM+), CD4+ T cells (CD3+ CD4+), CD8+ T cells (CD3+ CD8+), B cells (CD19+) and myeloid cells (CD11b+). Cells were also analyzed for surface expression of PD-1, PD-L1, and PD-L2, and tSNE plots were generated to evaluate their expression patterns across cell types. PD-1 was largely restricted to CD4+ and CD8+ T cells, while PD-L1 and PD-L2 were expressed by the tumor and myeloid cell populations.

Stimulatory OX40/OX40L, GITR/GITRL Expression



Dissociated tumor cells from a lung cancer patient were analyzed by flow cytometry for tumor cells (EpCAM+), CD4+ T cells (CD3+ CD4+), CD8+ T cells (CD3+ CD8+), B cells (CD19+) and myeloid cells (CD11b+). Cells were also analyzed for surface expression of OX40/OX40L or GITR/GITRL, and tSNE plots were generated to evaluate their expression patterns across cell types. OX40L and GITRL expression was not observed in any cell populations within the tumor microenvironment. OX40 and GITR, on the other hand, were largely restricted to CD4+ T cell compartment.

TIGIT/PVRL2 Expression - Matched Samples



Matched dissociated tumor cells (DTCs) and peripheral blood mononuclear cells (PBMCs) from a lung cancer patient were analyzed by flow cytometry for tumor cells (EpCAM+), CD4+ T cells (CD3+ CD4+), CD8+ T cells (CD3+ CD8+), B cells (CD19+) and myeloid cells (CD11b+). Cells were also analyzed for surface expression of TIGIT, PVR (CD155) and PVRL2 (CD112). PVR expression was patient-dependent and largely restricted to tumor cells. tSNE plots were generated to evaluate the expression of TIGIT and PVRL2. TIGIT was restricted to CD4+ and CD8+ T cells. Additionally, the percentage of TIGIT+ cells was higher in DTCs compared to PBMCs. PVRL2 expression, on the other hand, was primarily observed on tumor cells and myeloid cells in both DTCs and PBMCs.

SUMMARY

- **Unbiased sequencing analysis revealed extensive expression of immunomodulatory receptors**
  - PD1/PDL1/PDL2, TIGIT/PVR/PVRL2, Tim3/Galectin9, and Lag3 are highly expressed
  - OX40/OX40L, 4-1BB/4-1BBL, and GITR/GITRL pairs are expressed
- **PD1/PDL1/PDL2 displays dynamic expression within the tumor microenvironment**
  - PD1 expression is largely restricted to CD4+ and CD8+ T cell subsets, with some B cell expression observed
    - PDL1 and PDL2 are expressed by the tumor and myeloid cell populations
- **Co-stimulatory receptors, but not their cognate ligands, are expressed within the tumor microenvironment**
  - OX40 and GITR are primarily expressed by CD4+ T cells
  - OX40L and GITRL expressed was undetectable on any cell populations
- **TIGIT and PVRL2 expression is restricted to distinct cellular subsets in DTCs and PBMCs**
  - TIGIT was primarily observed on CD4+ and CD8+ T cells
  - PVRL2 expression was restricted to tumor and myeloid cells