PVRIG, a novel T cell checkpoint, is preferentially expressed in TLS on stem-like memory T cells, potentially inhibiting their expansion

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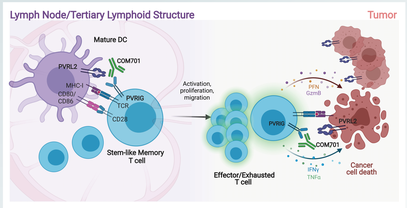
Abstract

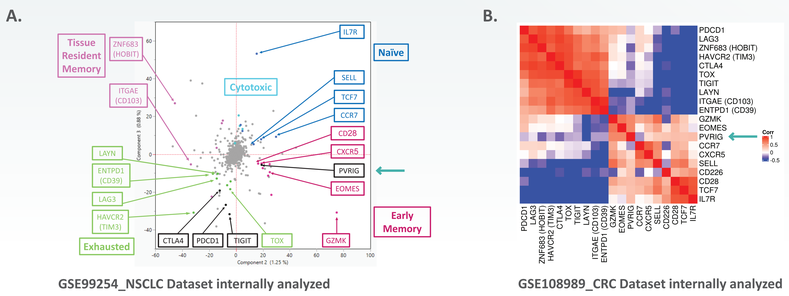
Background: Tertiary lymphoid structures (TLS) recently emerged as an intra-tumoral niche with a predictive value for cancer immunotherapy responses. LAMP3$^+$ DCs in the TLS were shown to interact with and support the differentiation of stem-like CD8$^+$ (Tscm) into effector-like cells, that then expand in the tumor micro-environment (TME) and may exert anti-tumor responses. We have previously shown that PVRIG is expressed on Tscm and exhausted T cells, but has a unique dominant expression on Tscm cells, while PVRL2 is abundantly expressed across DC types [1]. In this study, we further explored the expression of DNAM-1 axis genes in the TME, and assessed the immune response in the blood and TME of cancer patients treated with COM701 (anti-PVRIG antibody) monotherapy or COM701+nivolumab combination therapy.

Methods: MERFISH technology was employed to detect the expression of 350 distinct mRNA transcripts at sub-cellular resolution in CRC sections. Extensive omics profiling was performed on pre- and on-treatment biopsies from patients in the COM701 and COM701+nivolumab Phase-1 clinical study (NCT03667716).



1. Alteber et al., P252, SITC 2021, http://dx.doi.org/10.1136/jitc-2021-SITC2021.252

PVRIG uniquely clusters with early differentiated/Tscm genes

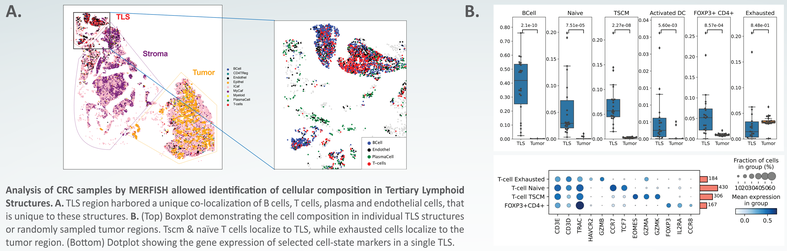


GSE99254_NSCLC Dataset internally analyzed

GSE108989_CRC Dataset internally analyzed

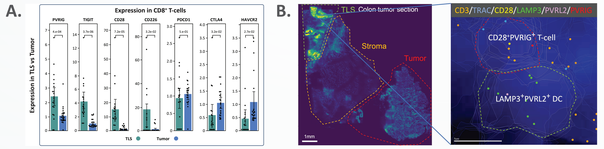
A. Unsupervised PCA was performed on the scRNA expression matrix of TME CD8$^+$ T cells, which includes all variable genes. Using cells as features and genes as entries, co-expression pattern among genes known to be expressed on naïve, memory, and exhausted CD8$^+$ T cells is shown. Nine datasets were analyzed, representative NSCLC dataset is shown. **B.** scRNA-seq datasets (CRC, NSCLC, HNSCC, Melanoma, Liver cancer, n=13) were analyzed for the co-expression pattern of 19 genes, including genes known to be expressed on naïve (TCF7, IL7R, SELL), memory (GZMK, EOMES), and exhausted (PDCD1, LAG3, HAVCR2) CD8$^+$ T cells. Representative dataset of CRC is presented.

Spatial transcriptomic analysis of TLS regions shows enrichment of Tscm and dendritic cells, while exhausted cells localize to the tumor



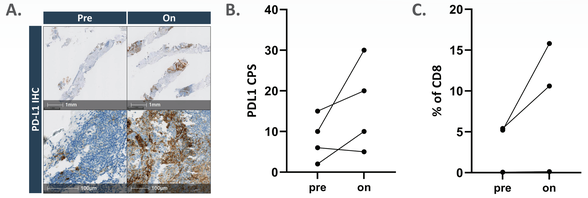
Analysis of CRC samples by MERFISH allowed identification of cellular composition in Tertiary Lymphoid Structures. A. TLS region harbored a unique co-localization of B cells, T cells, plasma and endothelial cells, that is unique to these structures. **B.** (Top) Boxplot demonstrating the cell composition in individual TLS structures or randomly sampled tumor regions. Tscm & naïve T cells localize to TLS, while exhausted cells localize to the tumor region. (Bottom) Dotplot showing the gene expression of selected cell-state markers in a single TLS.

PVRIG and other genes of the DNAM-1 axis are dominantly expressed in TLS region



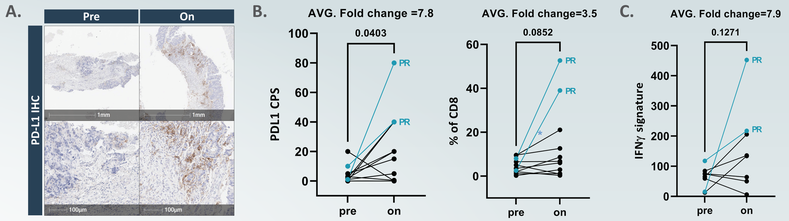
DNAM-1 axis is dominantly expressed in TLS region. A. Genes of the DNAM-1 axis, PVRIG, TIGIT, and CD226, show dominant expression in TLS region, whereas other immune checkpoints, such as PD-1, CTLA4, and TIM3 are expressed more in the tumor bed. **B.** CD28$^+$CD8$^+$ T cell expressing PVRIG intimately interacts with LAMP3$^+$PVRL2$^+$ DC in the TLS of a CRC patient.

COM701 monotherapy induces TME immune modulation in patients with ovarian cancer



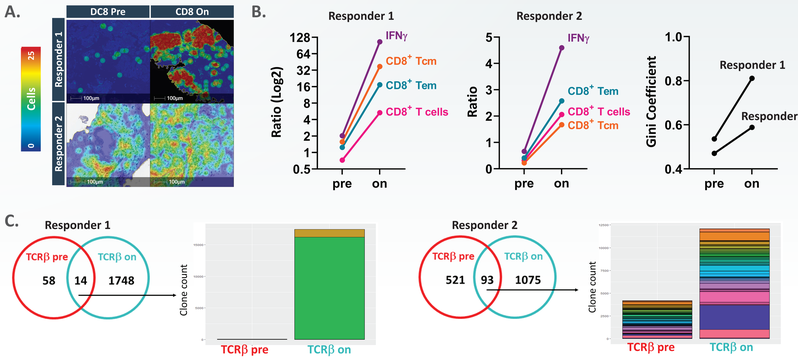
Increased TME immune activation following COM701 monotherapy in ovarian cancer patients. A. Monotherapy with COM701 increased PD-L1 expression (IHC, SP263) in ovarian cancer. **B. and C.** An increase in PD-L1 CPS score (SP263) in 3 out of 4 patients, and in CD8$^+$ cells percentages (IF, C8/144B, quantification with HALO) in 2 out of 3 patients. All panels compare on-treatment (between cycle 2 and 3) to pre-treatment biopsies.

COM701+nivolumab combination induces TME immune modulation in patients with MSS-CRC



Increased TME Immune activation following COM701+nivolumab combination in MSS CRC patients. A. PD-L1 expression (IHC, 28-8) is induced by combination therapy. **B.** An increase in PD-L1 CPS score (28-8) in 9 out of 13 patients, and CD8 quantification (IHC, C8 /144B) in 7 out of 11 patients biopsies treated with COM701+nivolumab. **C.** An increase in IFNg mRNA signature in 5 out of 8 patients in on-treatment biopsies compared to pre-treatment. All panels compare on-treatment (between cycle 2 and 3) to pre-treatment biopsies. PR: patient with partial response per RECIST.

Extensive TME modulation in MSS-CRC patients partially responding to COM701+ nivolumab



Increased TME immune activation and TCR clonality in patients with MSS CRC with PR to COM701+nivolumab combination therapy. Pre- and on-treatment biopsies from COM701+nivolumab treated patients with MSS-CRC were subjected to Personalis®, ImmunoID NeXT analysis **A-B.** Increased immune infiltration and activation in the TME post COM701+nivolumab therapy. **C.** Increased number of clones and increased clonal expansion as was determined by Gini coefficient in the TME post COM701+nivolumab therapy. In both patients the most expanded clone was present prior to treatment initiation.

Conclusion

By leveraging spatial and scRNA transcriptomics, we identified PVRIG$^+$CD28$^+$CD8$^+$ T cells predominantly localized within TLS, interacting with PVRL2$^+$LAMP3$^+$ DCs. PVRIG blockade could therefore enhance the differentiation and expansion of stem-like CD8 T cells into effector-like cells. Accordingly, translational data shows increased T cells infiltration and immune activation in response to COM701 or COM701+nivolumab, in patients with less-inflamed indications, normally not responsive to approved CPI treatment.