Elucidating the Mechanism of Action of the Novel Immune Checkpoint PVRIG: Insights from Single-Cell and High-Resolution Spatial Transcriptomics

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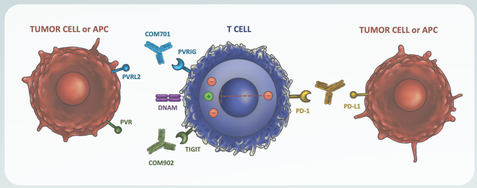
Introduction

Background: Cancer immunotherapy has emerged as a groundbreaking treatment for some patients with cancer, yet its applicability and effectiveness remain limited. Therefore, there is a significant unmet medical need to identify new checkpoints and checkpoint inhibitors to address immunotherapy resistance.

Methods and finding: Based on evolutionarily conservation of genomic exon arrangement we have discovered PVRIG as a novel T-cell checkpoint and are currently evaluating COM701, a potential first in class anti-PVRIG antibody in clinical studies. While exploring its unique biology, we found that PVRIG is not accurately detected in scRNA-seq and other types of assays that utilize the GENCODE gene model, thus hindering its research. We generated a corrected reference genome and demonstrated that by employing these changes we could capture accurate PVRIG expression levels. Using this reference, we conducted comprehensive analysis of scRNA-seq datasets and devised a unique dimension reduction strategy to highlight differences between delicate T-cell states. We found that PVRIG is uniquely expressed in stem like T-cell population (Tscm), which have strong proliferative capacity, while all other T-cell checkpoints targeted by approved immunotherapies and TIGIT were found more abundantly on exhausted cells. Using MERFISH, we analyzed tertiary lymphoid structures (TLS), which hold predictive value for immunotherapy responses. We provide a detailed examination of DNAM-1 axis pathway activity in these regions and show that its members are preferentially found in TLS versus the tumor.

DNAM axis potential to be a game changer in the fight against cancer

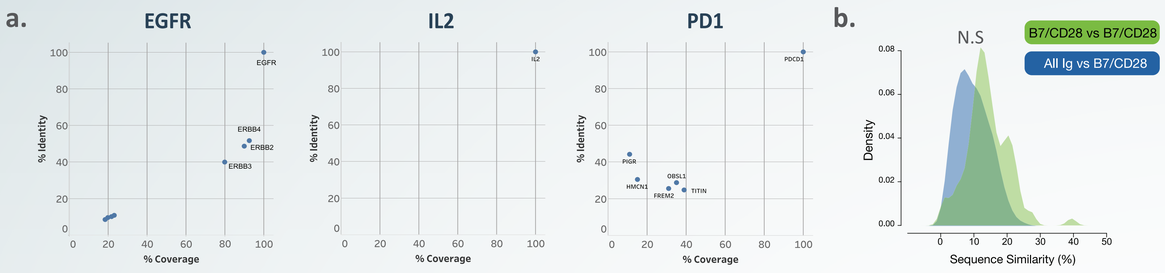


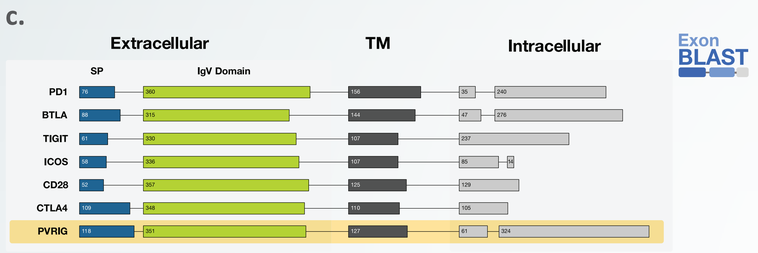
Support combination approach to overcome immunotherapy resistance

- PVRIG and TIGIT discovered by Compugen's discovery platform
- DNAM axis – two parallel and complementary inhibitory pathways (PVRIG & TIGIT)
- Potential intersection between PVRIG/TIGIT and PD-1 pathway

Discovery of PVRIG via evolutionarily conservation of genomic exon arrangement

Employing our unique computational algorithms to define new members of the B7/CD28 family we identified PVRIG, as a potential novel immune checkpoint.

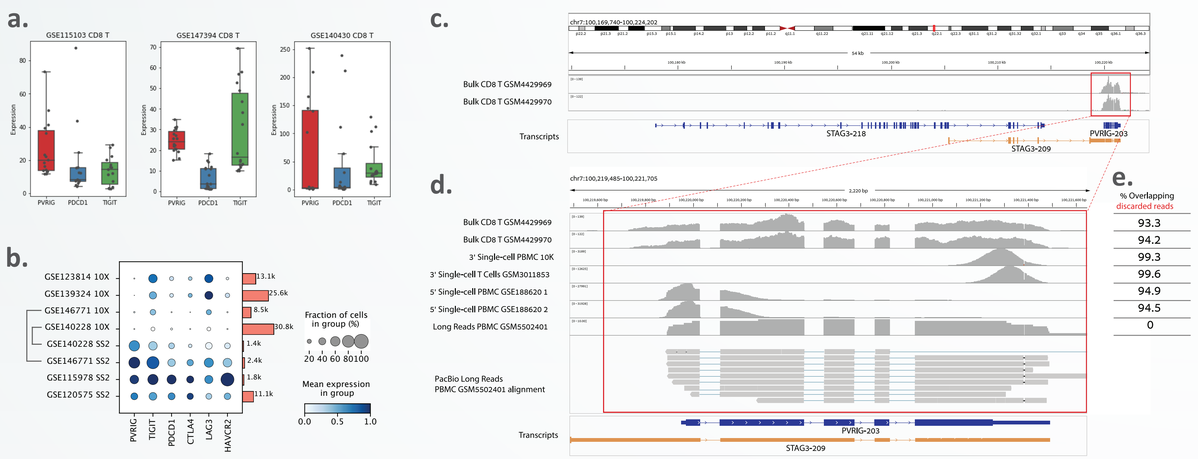




Identification of PVRIG using the Exon-Blast methodology. a. Plots illustrating the lower sequence conservation of immune-related genes (e.g., IL2 and PD1) compared to EGFR family members: This is indicative of evolutionary pressure exerted on immune genes. **b.** Histogram demonstrating that sequence similarity among B7/CD28 family members is not significantly greater than their similarity to Ig family genes: This further emphasizes the limited sequence conservation in immune genes. **c.** Output from the Exon-Blast algorithm reveals that exon size and arrangement are conserved among known immune checkpoints. This led to the discovery that PVRIG could potentially serve as one.

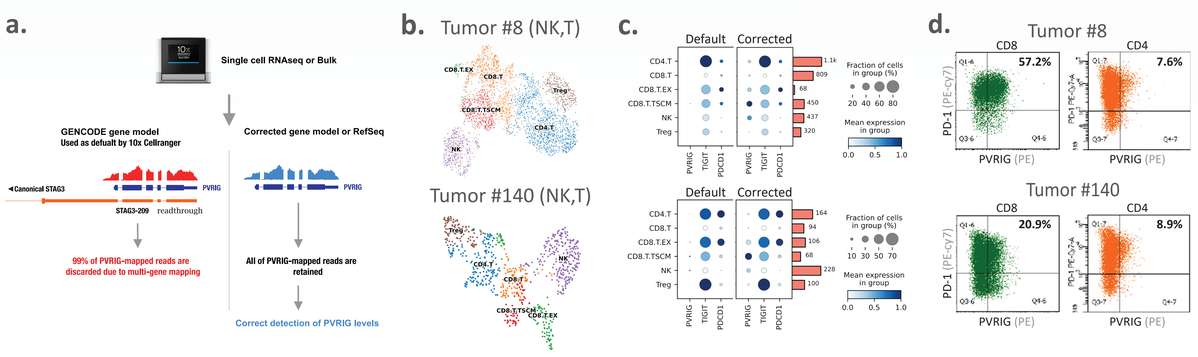
PVRIG is poorly detected in 10x Chromium data due to overlap with STAG3

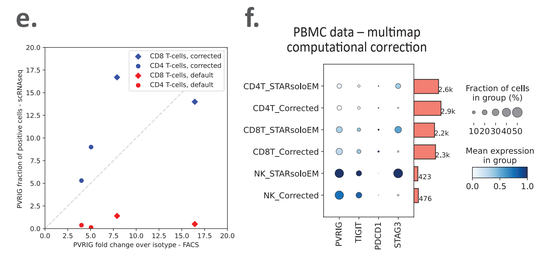
We noted that in 10x Chromium datasets, PVRIG is poorly detected due to multi-mapping of reads to STAG3-209, a read-through transcript with poor support levels that was described only once in the literature, and is not detected in 10x or PacBio long-read sequencing.



Unlike bulk and smart-seq PVRIG is poorly detected in 10x single cell data due to overlap with STAG3-209. a. Boxplots showing that PVRIG, TIGIT and PD-1 are expressed across several CD8 T-cell datasets as measured using bulk RNA-seq. GSE140430 isolated CD8 T-cells from TME, rest are from blood. **b.** DotPlot showing the expression of PVRIG and additional T-cell checkpoints in several Smart-seq2 (SS2) datasets (bottom) but PVRIG is not detected in 10x datasets (top). Line connect datasets from the same studies conducted with the two technologies. Bars indicate number of cells in each sample. **c.** The STAG3-PVRIG genomic locus. Histograms feature the distribution of reads in bulk RNA-seq data of isolated CD8 T-cells highlighting that PVRIG is expressed in such cells (red box). Gene structures appear below in blue. **d.** Closer view of PVRIG locus. Histograms highlight the distribution of reads in the indicated datasets. **e.** Fraction of reads that map to both PVRIG and STAG3-209 and are thus discarded.

Corrected gene model enables correct detection of PVRIG and validates expression in Tscm in ovarian tumor samples

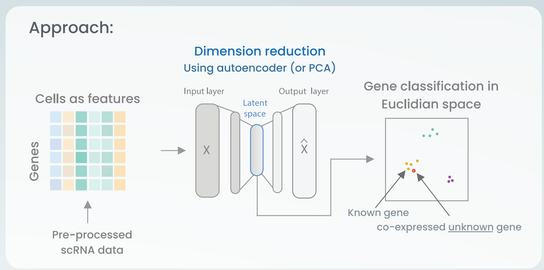




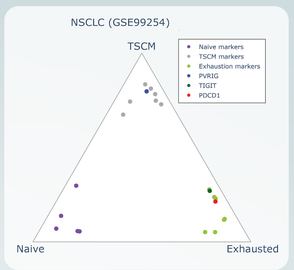
Corrected gene model enables the detection of PVRIG. a. Summarizing illustration; using the GENCODE gene model reads are discarded due to multi-mapping, following the correction of the gene model PVRIG expression is correctly measured. **b.** UMAPs showing the population distribution in two ovarian tumor samples subset for T and NK cells. **c.** DotPlot showing the expression of PVRIG and additional T-cell checkpoints in ovarian tumor samples processed using the default or corrected gene model subset for T and NK cells. Bars indicate number of cells in each sample. **d.** Matched FACS analysis showing the expression of PVRIG and PD-1 in CD8 or CD4 T-cells. **e.** Graph showing PVRIG-positive fraction as measured using FACS (X-axis) to its parallel in the scRNA-seq before and following the gene model correction. T-cells are sperated into CD4+ and CD8+ as indicated. **f.** DotPlot showing the expression of PVRIG, TIGIT, PD-1 and STAG3 in NK/T populations in the PBMC10K dataset processed using the corrected gene model or using STARsolo-EM method.

Gene model correction allows the study of PVRIG in tumor samples, highlighting unique association with Tscm cells

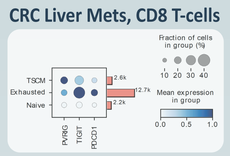
a. Unique outlook on gene clustering (vs cell states) to gain insights



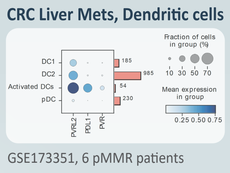
b. Analysis of CD8+ T-cells in NSCLC



c. CRC Liver Mets, CD8 T-cells



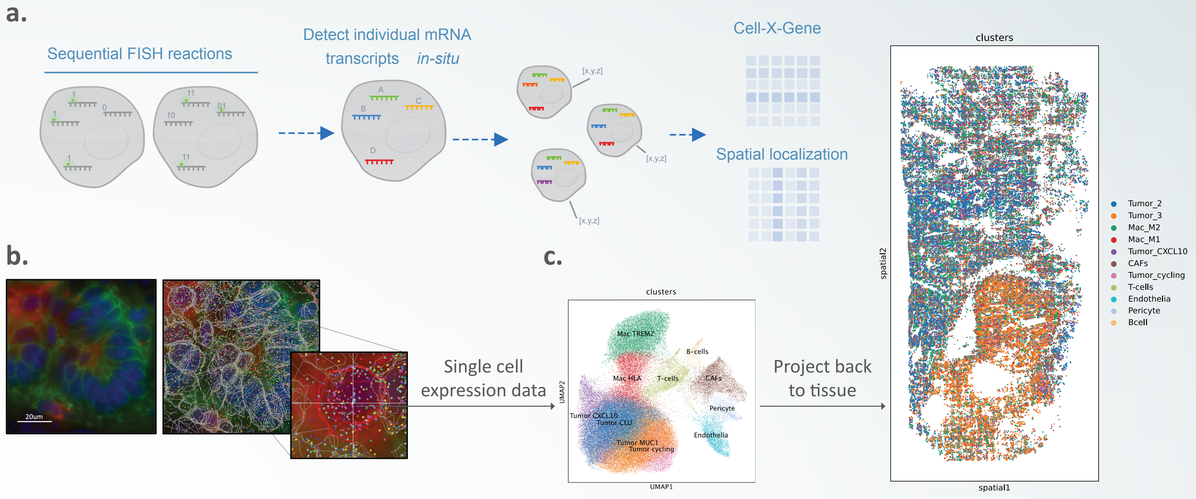
d. CRC Liver Mets, Dendritic cells



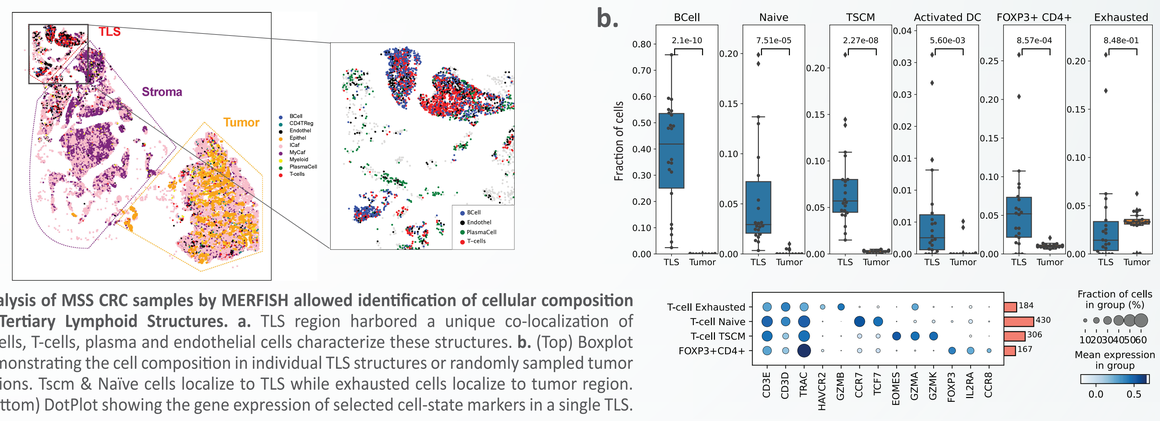
GSE173351, 6 pMMR patients

Analysis of scRNA data using the corrected model supported DNAM-1 MOA and differentiation of PVRIG expression vs TIGIT. a. Our approach to find co-expressed genes is based on generating custom dimension reduction using deep-learning approach or PCA. **b.** Ternary plot showing the proximity of PVRIG, TIGIT and PD1 (PDCD1) to cell-state centroids, showing PVRIG is associated with Tscm state while the other checkpoints are associated with an exhausted state. **c.** DotPlot showing the expression of PVRIG, TIGIT and PD-1 in CD8 cells isolated MSS CRC mets. PVRIG shows dominant expression on Tscm compared to TIGIT and PD1. **d.** DotPlot showing the expression of corresponding ligands in dendritic cells from the MSS CRC liver mets. Dominant PVRL2 expression is observed on DCs compared to PVR and PDL1.

MERFISH Technology

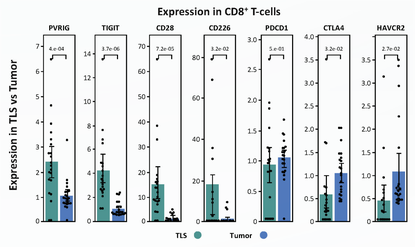


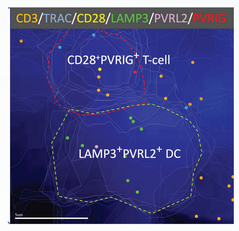
Spatial transcriptomic analysis of TLS regions shows enrichment of Tscm and dendritic cells while exhausted cells localize to the tumor



Analysis of MSS 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 characterize these structures. **b.** (Top) Boxplot demonstrating the cell composition in individual TLS structures or randomly sampled tumor regions. Tscm & Naïve cells localize to TLS while exhausted cells localize to 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 regions

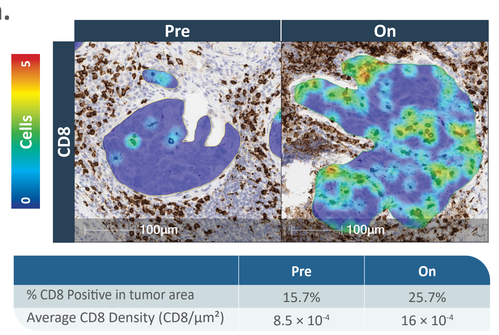




DNAM-1 axis is dominantly expressed in TLS regions. a. Member genes of the DNAM-1 axis, PVRIG, TIGIT, CD226, show dominant expression in TLS regions, whereas other immune checkpoints, such as PD-1, CTLA4, TIM3 are expressed more in the tumor bed. **b.** CD28+ CD8 T-cell expressing PVRIG intimately interacts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

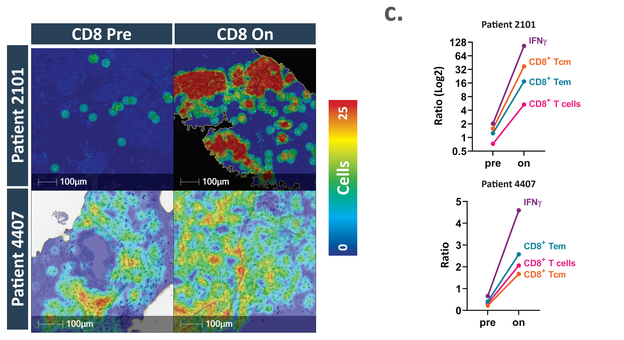
Translational data from PVRIG inhibition in clinical studies demonstrates immune activation

Increased CD8+ T cell infiltration post COM701 and nivolumab treatment in platimun resistant ovarian carcinoma



	Pre	On
% CD8 Positive in tumor area	15.7%	25.7%
Average CD8 Density (CD8/µm²)	8.5×10^{-4}	16×10^{-4}

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



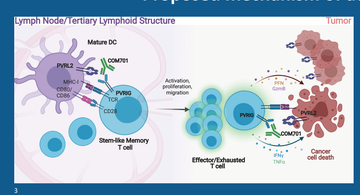
a. The expression pattern of PVRIG on Tscm in Ovarian cancer correlates with clinical data showing increased CD8 IHC staining in patient with high grade serous carcinoma who had a partial response following treatment, treated with COM701 and nivolumab, who received 7 prior lines of anti cancer treatment (including nivolumab). CD8 intensity was calculated using Halo Density heatmap algorithm.[1] **b-c.** The expression pattern on PVRIG on Tscm in CRC liver mets correlates with translational data showing increased immune infiltration and activation in the TME post COM701+nivolumab therapy in MSS CRC patients with liver mets, with PR following treatment.[2]

References: 1. Yeku O et al, ESMO-IO 2022, **2.** Ophir, E, et al, SITC 2022, **3.** Alteber et al 2021, SITC

CONCLUSION

PVRIG, which was discovered using our computational platform is a novel immune checkpoint. Through refinements of technical elements in scRNA analysis and development of algorithmic approaches to study gene co-expression we showed that PVRIG is associated with Tscm state. While harnessing the MERFISH technology we demonstrated that PVRIG+CD28+ CD8 Tscm predominantly localize within TLS, interacting with PVRL2+LAMP3+ DCs. PVRIG blockade could therefore enhance the differentiation and expansion of Tscm, driving T-cells immunity also in less inflamed tumors, as supported by initial translational data.

Proposed mechanism of action for COM701 (anti-PVRIG)



- Based on our computational analysis and experimental data we believe that PVRIG+ Tscm interact with PVRL2+ DCs in the LN or TLS
- Blocking PVRIG is potentially unique in generating new waves of T cells to infiltrate the TME
- Hence, COM701 may sensitize cold tumors to PD-1/TIGIT