

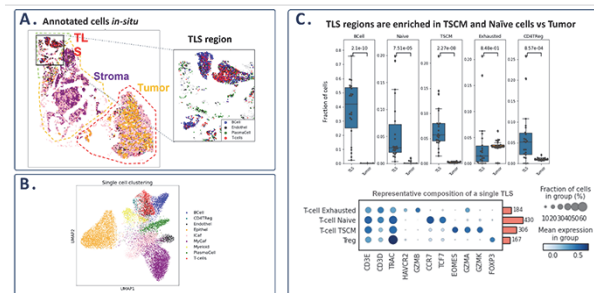
**PVRIG, A NOVEL T CELL CHECKPOINT, IS PREFERENTIALLY EXPRESSED IN TLS ON STEM-LIKE MEMORY T CELLS, POTENTIALLY INHIBITING THEIR EXPANSION**

<sup>1</sup>Zoya Alteber, <sup>1</sup>Roy Granit, <sup>1</sup>Gady Cojocar, <sup>1</sup>Amit Novik, <sup>1</sup>Niv Sabath, <sup>1</sup>Assaf Wool, <sup>1</sup>Yossef Klinger, <sup>2</sup>Yu Liang, <sup>3</sup>Natalia Petrenko, <sup>3</sup>Jiang He, <sup>1</sup>Pierre Ferre, <sup>1</sup>Yaron Turpaz, <sup>1</sup>Zurit Levin, <sup>1</sup>Eran Ophir\*. <sup>1</sup>Compugen Ltd., Holon, Israel; <sup>2</sup>Compugen USA Inc., South San Francisco, CA, United States; <sup>3</sup>Vizgen Inc., Cambridge, MA, United States

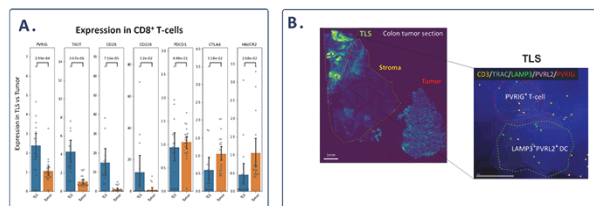
**Background** Tertiary lymphoid structures (TLS) recently emerged as an intra-tumoral niches of immune-cell aggregates with a predictive value for cancer immunotherapy responses.<sup>1</sup> LAMP3<sup>+</sup>DCs in the TLS were shown to interact and support the differentiation of stem-like CD8 T-cells into effector-like cells, that then expand in the tumor micro-environment (TME) and may exert anti-tumor responses.<sup>2</sup> We investigated the expression of DNAM-1 axis genes: PVRIG, TIGIT, DNAM-1 and their ligands PVRL2 and PVR in the TME.<sup>3</sup>  
**Methods** MERFISH technology was employed to detect the expression of 350 distinct mRNA transcripts at sub-cellular resolution in CRC sections. Publicly available TME scRNA-seq datasets were analyzed for expression of PVRIG and PVRL2 across immune populations and validated by flow-cytometry. An extensive omics profiling was performed for patients with pre- and on-treatment biopsies from COM701 (anti-PVRIG antibody) and COM701+nivolumab Phase-1 study (NCT03667716).

**Results** Spatial distribution of gene transcripts allowed identifying localization of stem-like T-cells in TLS regions of two CRC patients (figure 1, p<0.001). While, CTLA-4, PD-1, and TIM3 were mainly expressed by tumor infiltrating T cells, PVRIG and other genes of DNAM-1 axis were also largely expressed in tumor bed, and even more intensely in TLS (p<0.05, figure 2). Furthermore, high resolution unsupervised scRNA gene co-expression analysis in the TME further validated that while PD-1 is strongly correlated with TIM3, CTLA-4, and other markers of exhausted T-cells, PVRIG uniquely clusters with markers of stem-like T-cells. The PVRIG protein expression was increased on CD28<sup>+</sup> stem-like T-cells across indications (figure 3). RNA and protein expression data identified PVRL2, PVRIG ligand, preferentially expressed across DC-subtypes compared to PD-L1 and PVR (figure 4). PVRIG blockade could therefore enhance memory T-cell activation by DCs, resulting in their increased expansion and differentiation. Accordingly, COM701 monotherapy induced CD8<sup>+</sup> T-cell numbers and immune activation in the TME of ovarian cancer patient (figure 5). Moreover, MSS-CRC patient with partial response to COM701+nivolumab, demonstrated an increase in TCR numbers, clonality, T-cell infiltration and activation in the TME (figure 6). Finally, preliminary analysis of serum from two patients clinically responding to COM701+nivolumab (RECIST criteria), revealed induction of activated-DC markers, compared to non-responders (figure 7).

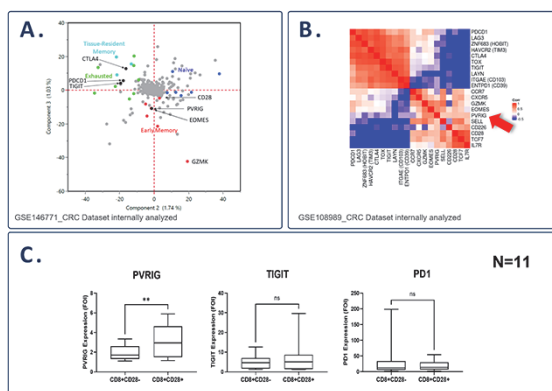
**Conclusions** By leveraging spatial and scRNA transcriptomics, we identified PVRIG<sup>+</sup>CD8<sup>+</sup>T-cells predominantly localized within TLS, interacting with PVRL2<sup>+</sup>LAMP3<sup>+</sup>DCs. PVRIG blockade could therefore enhance the differentiation and expansion of stem-like CD8<sup>+</sup> T-cells into effector cells (figure 8). Accordingly, early clinical data shows increased T-cells infiltration and immune activation in patients treated with COM701 or COM701+nivolumab.



**Abstract 504 Figure 1** Analysis of CRC samples by Vizgen allowed identification of Tertiary Lymphoid Structures. A. TLS region harbored a unique co-localization of B-cells, T-cell, Plasma and endothelial cells that are unique to these structures. B. UMAP showing the clustering of cell populations into distinct cell types based on MRFISH mRNA readout. C. (Top) Boxplot demonstrating the cell composition in individual TLS structures or randomly sampled tumor regions. Tscm & naive T cells localize to TLS while exhausted T cells localize to tumor region. (Bottom) Dotplot showing the gene expression of selected cell-state markers in a single TLS.

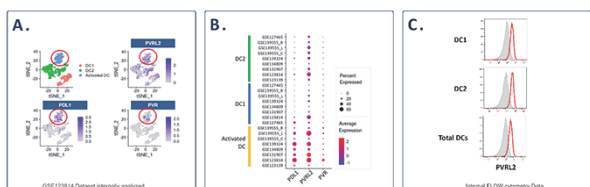


**Abstract 504 Figure 2** DNAM-1 axis is dominantly expressed in TLS region. A. genes of the DNAM-1 axis, PVRIG, TIGIT, CD226, show dominant expression in TLS region, whereas other immune checkpoints, such as PD-1, CTLA4, TIM3 are expressed in the tumor bed. B. PVRIG expressing T cells intimately interacts with LAMP3<sup>+</sup> PVRL2<sup>+</sup> DC in the TLS of CRC patient.

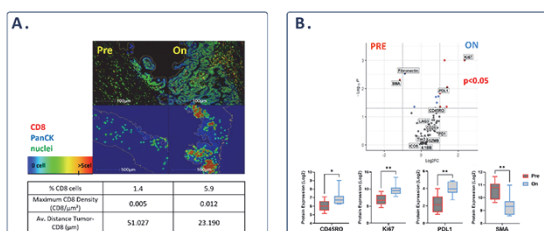


**Abstract 504 Figure 3** PVRIG clusters with early differentiation/memory genes in the TME, unlike other immune checkpoints that cluster with exhausted genes, in CD8<sup>+</sup> T cells. A. Unsupervised PCA analysis was performed on a scRNA expression matrix of TME CD8<sup>+</sup> T cells, which includes all variable genes. Using cells as features and genes as entries, hierarchical co-expression pattern among genes known to be expressed on naive, memory, and exhausted CD8<sup>+</sup> T-cells was performed. B. scRNA-Seq datasets were analyzed for co-expression pattern among 19 genes, including genes known to be expressed on naive (TCF7, IL7R, SELL), memory (GZMK, EOMES), and

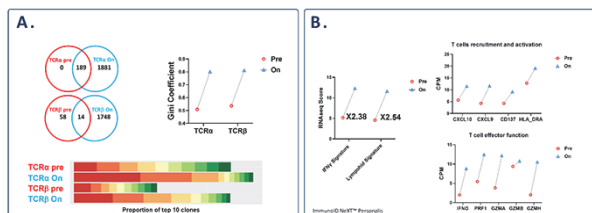
exhausted (PDCD1, LAG3, HAVCR2) CD8 T cells. Average gene-gene correlation over all datasets was calculated. Representative CRC dataset of n=13 (CRC, NSCLC, HNSCC, Melanoma, Liver cancer) is presented. C. Samples (n=11) of CRC, ovarian and bladder cancer were dissociated to single cell suspensions and analyzed for gene expression by flow-cytometry. Paired T-test was used to compare between PVRIG expression among cell populations.



**Abstract 504 Figure 4** PVRL2 is dominantly expressed on dendritic cells in the TME. A. tSNE map depicting the expression profile of PVR/PVRL2/PDL1 in major dendritic cell subsets in Basal Cell Carcinoma patients. B. Dot plots showing the percent of cells and average level of expression of PVR/PVRL2/PD-L1 in major dendritic cell subsets across multiple scRNA-seq cancer datasets. C. PVRL2 protein expression across DC subsets in a representative ovarian cancer sample analyzed by flow-cytometry.

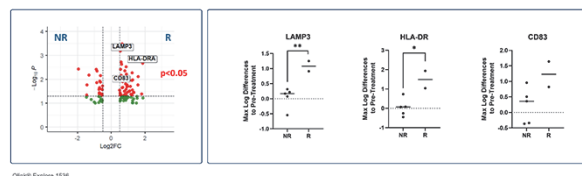


**Abstract 504 Figure 5** COM701 Monotherapy induced immune activation in the TME of patient with ovarian cancer (radiologically defined as PD). Pre- and on-treatment biopsies from COM701 (anti-PVRIG antibody) treated patient with ovarian cancer were subjected to GeoMx® Immune Protein Assays, ROI selection was performed using DAPI, and mAbs detecting PanCK, CD8 and CD68. A. CD8 distribution in the TME post COM701 monotherapy. B. Protein expression in CD8 regions as was detected with Nanostring, DSP in the TME post COM701 monotherapy.

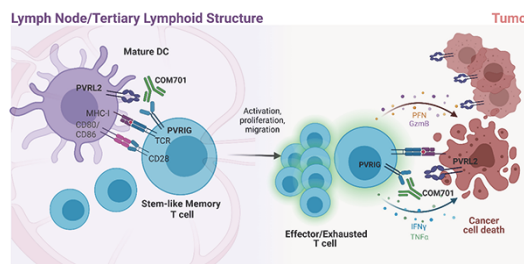


**Abstract 504 Figure 6** Increased TME immune activation and TCR clonality in patient with MSS CRC with PR to COM701+nivolumab combination therapy. Pre- and on-treatment biopsies from COM701 (anti-PVRIG antibody)+ nivolumab treated patient with MSS-CRC were subjected to Personalis®, Immunoid NeXT analysis A. Increased number of clones and increased clonal expansion as was determined by Gini coefficient in the TME post COM701+nivolumab therapy. B. Increased

immune infiltration and activation in the TME post COM701+nivolumab therapy



**Abstract 504 Figure 7** Combination of COM701+nivolumab Induced Markers of Activated DCs in Serum of Two Responding Patients. Serum of 7 patients from the nivolumab+COM701 dose escalation arm, were analyzed using Olink Explore 1536. For each patient, the maximal difference of log2 expression between all on-treatment time points and the pre-treatment value was calculated for each protein. Maximal log2 differences were compared by Student's t-test, with patients grouped based on response, RECIST criteria (responders (R): CR+PR vs. non responders (NR): SD+PD). Out of 10 proteins most significant for on-treatment up-regulation in responders, 3 are markers of activated DCs (LAMP3 ,HLA-DR and CD83).



**Abstract 504 Figure 8** Summary. PVRIG-expressing stem-like memory T cells in LNs and TLS of cancer patients, are interacting PVRL2+DCs. PVRIG blockade could therefore enhance the differentiation and expansion of stem-like memory CD8 T-cells into effector cells that proliferate and infiltrate tumor. In the tumor bed, PVRIG blockade could further potentiate anti-tumor responses by breaking the interaction between PVRIG+T cells and PVRL2-expressing tumor cells.

REFERENCES

- Schumacher TN, Thommen DS. Tertiary lymphoid structures in cancer. *Science*. 2022;**375**(6576):eabf9419
- Magen A, Hamon P, Fiaschi N, Troncoso L, Humblin E, D'souza D, Dawson T, Park MD, Kim J, Hamel S, Buckup M. Intratumoral mregDC and CXCL13 T helper niches enable local differentiation of CD8 T cells following PD-1 blockade. *bioRxiv*. 2022
- Alteber Z, Kotturi MF, Whelan S, Ganguly S, Weyl E, Pardoll DM, Hunter J, Ophir E. Therapeutic targeting of checkpoint receptors within the DNAM1 axis. *Cancer Discovery*. 2021;**11**(5):1040–51.

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