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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Background
Tertiary lymphoid structures (TLS) recently emerged as an intra-tumoral niche of immune-cell aggregates with a predictive value for cancer immunotherapy responses. LAMP3+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. We investigated the expression of DNAM-1 axis genes: PVRIG, TIGIT, CD226, and their ligands PVRL2 and PVR in the TME.

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+ 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+ 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+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 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 4  PVRL2 is dominantly expressed on dendritic cells in the TME. A. tSNE map depicting the expression profile of PVR/PVRL2/PD-L1 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-PVRL2 antibody) treated patient with ovarian cancer were subjected to GeoMx® Immune Protein Assays, ROI selection was preformed 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-PVRL2 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.

REFERENCES