CD161+ T_{RM} cells counteracts HPV-positive oropharyngeal cancer immunotherapy outcomes

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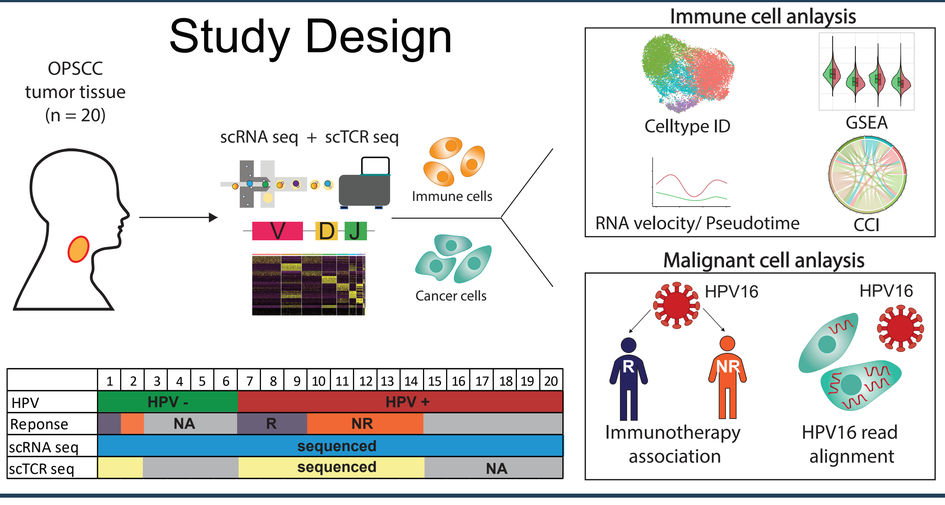
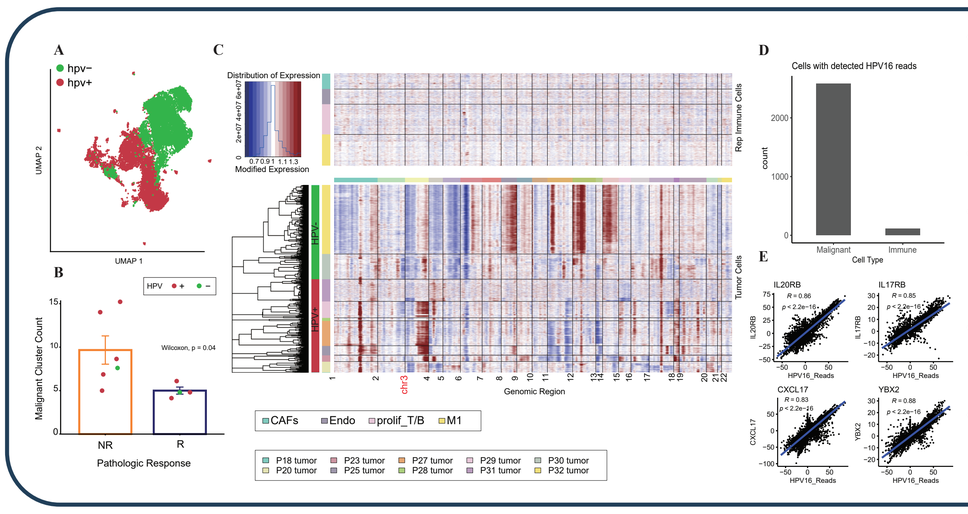
Highlights

- Transcriptional diversity of malignant cells negates immunotherapy response in oropharyngeal squamous cell carcinoma (OPSCC).
- HPV infection promotes differentiation of CD4+ folicullar helper T cells, enhancing antitumor activity.
- In HPV-positive OPSCC, anti-tumor effect is dampened in terms of TLS activity and T cell effector functions specifically in the tumor resident memory T cells (Trm) with elevated *KLRB1* (encoding CD161).
- Computational and experimental validation confirm that density of CD161+ Trm inversely correlates with immunotherapy efficacy in the HPV-positive OPSCC.

Study Design

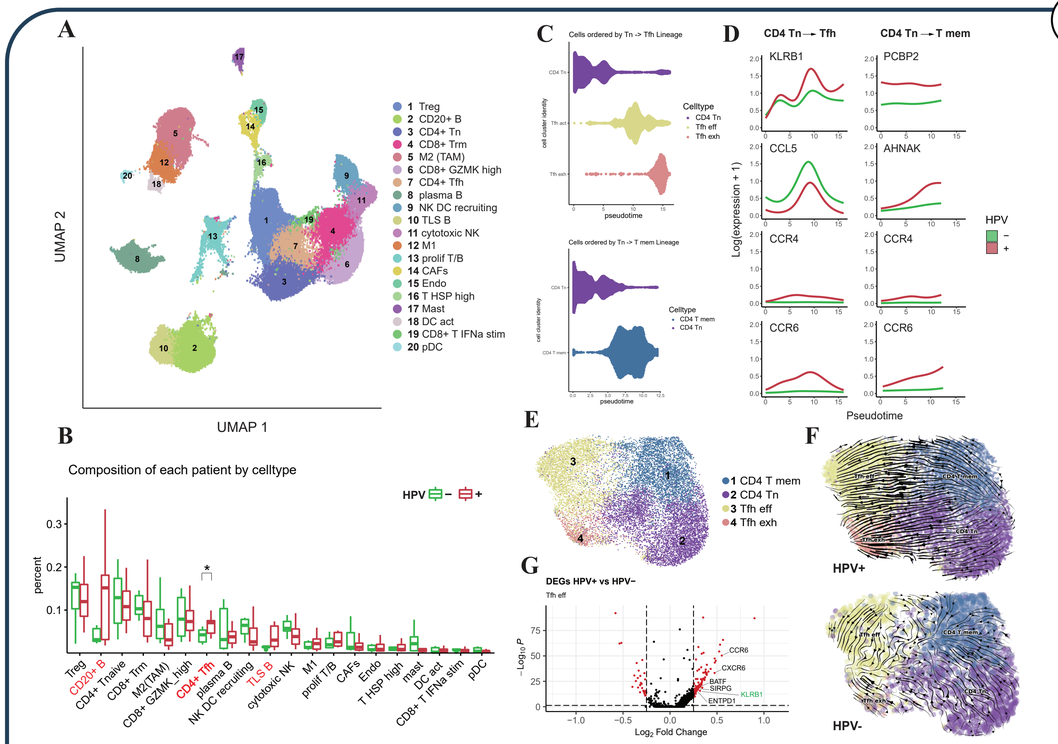


Malignant cells show transcriptome difference due to viral infection



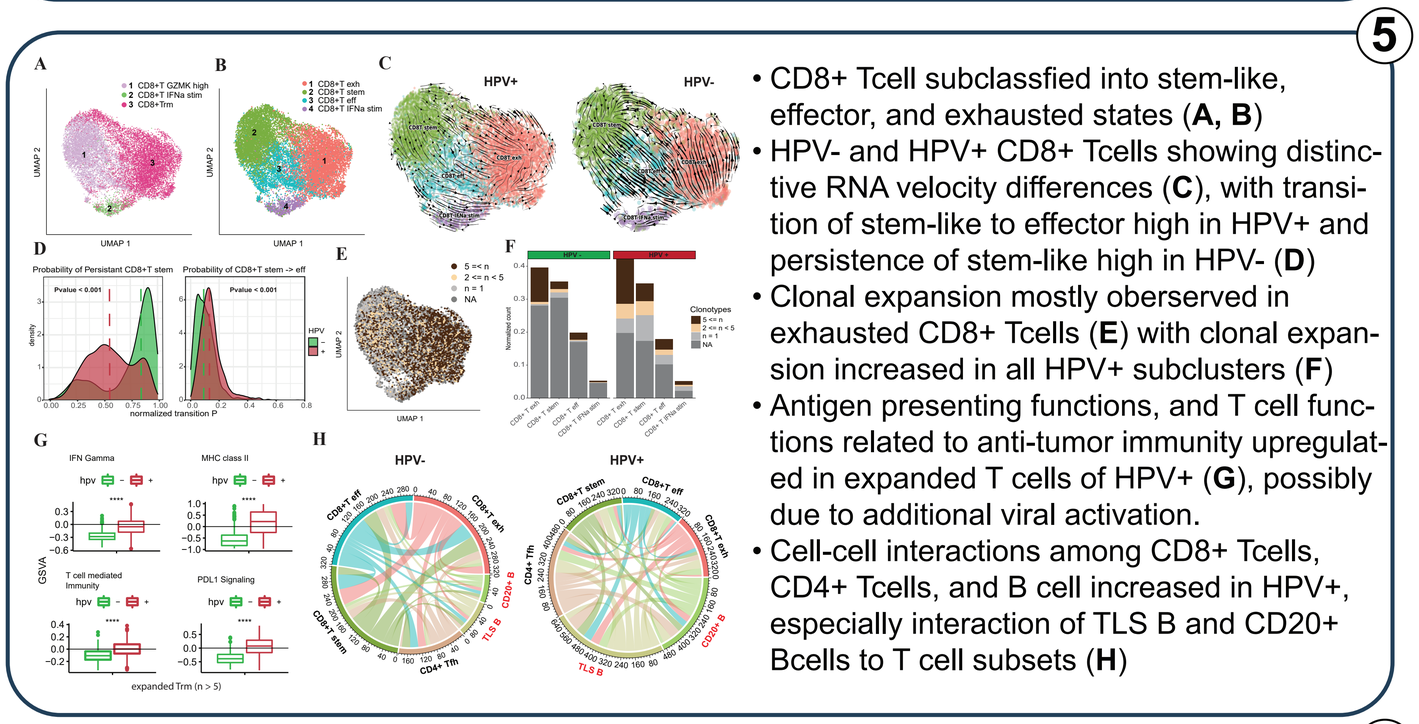
- Analysis of 8 HPV+ and 2 HPV- malignant cells show global transcriptome difference due to viral infection (**A**)
- Low transcriptome diversity predicts Immunotherapy response (**B**)
- broad copy number variations (CNV) in HPV- compared to HPV+, amplification in chr3 observed in most HPV+ samples (**C**)
- aligned HPV16 genome reads detected mostly in malignant cells compared to immune cells (**D**)
- Distinctive genes show correlations with HPV16 genome reads (viral titration, **E**)

20 immune/stromal cells identified (A). CD20+B, foillular helper T cells (CD4+ Tfh), and TLS B cells upregulated in HPV+ (B)



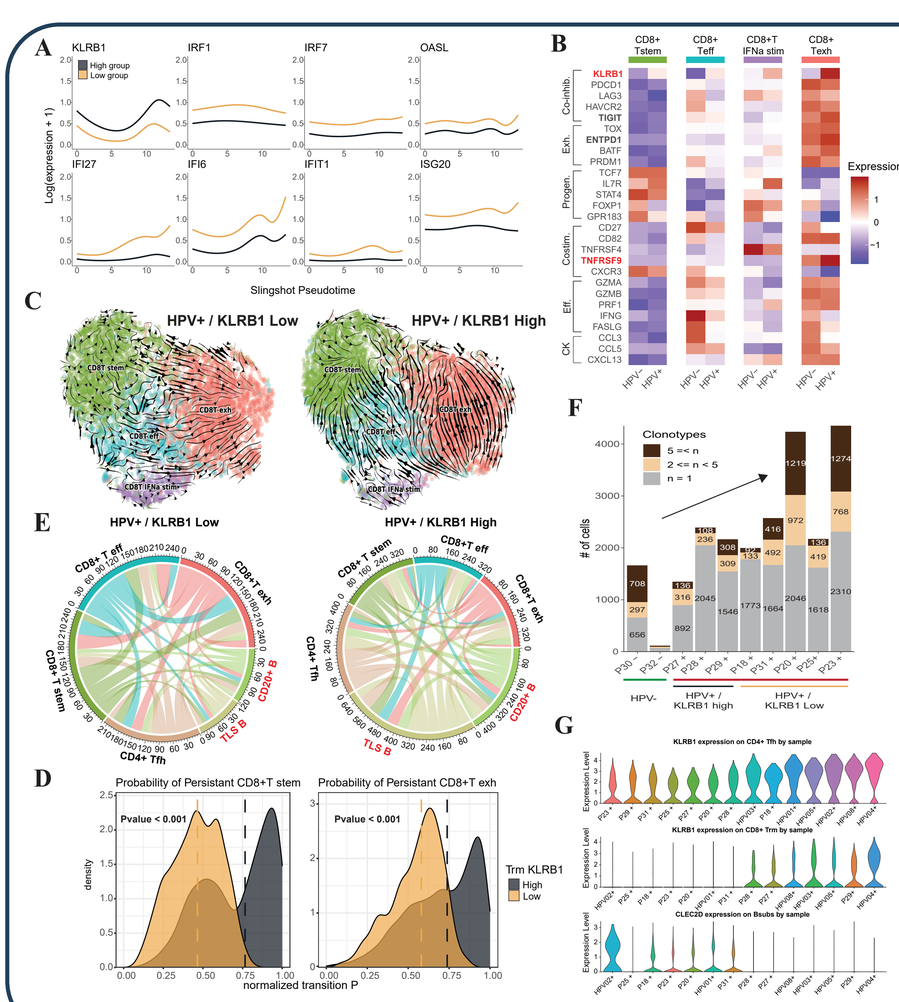
- Distinctive genes showing temporal dynamics along two pseudotime lineages (Tn->Tm, Tn->Tfh) (**C,D**) in CD4+ T cell subsets (**E**)
- Tfh differntiation prominent in HPV+ (**F**), distinctive genes upregulated in Tfh eff (**G**)

CD8+ Tcell subclassfied into stem-like, effector, and exhausted states (A, B)



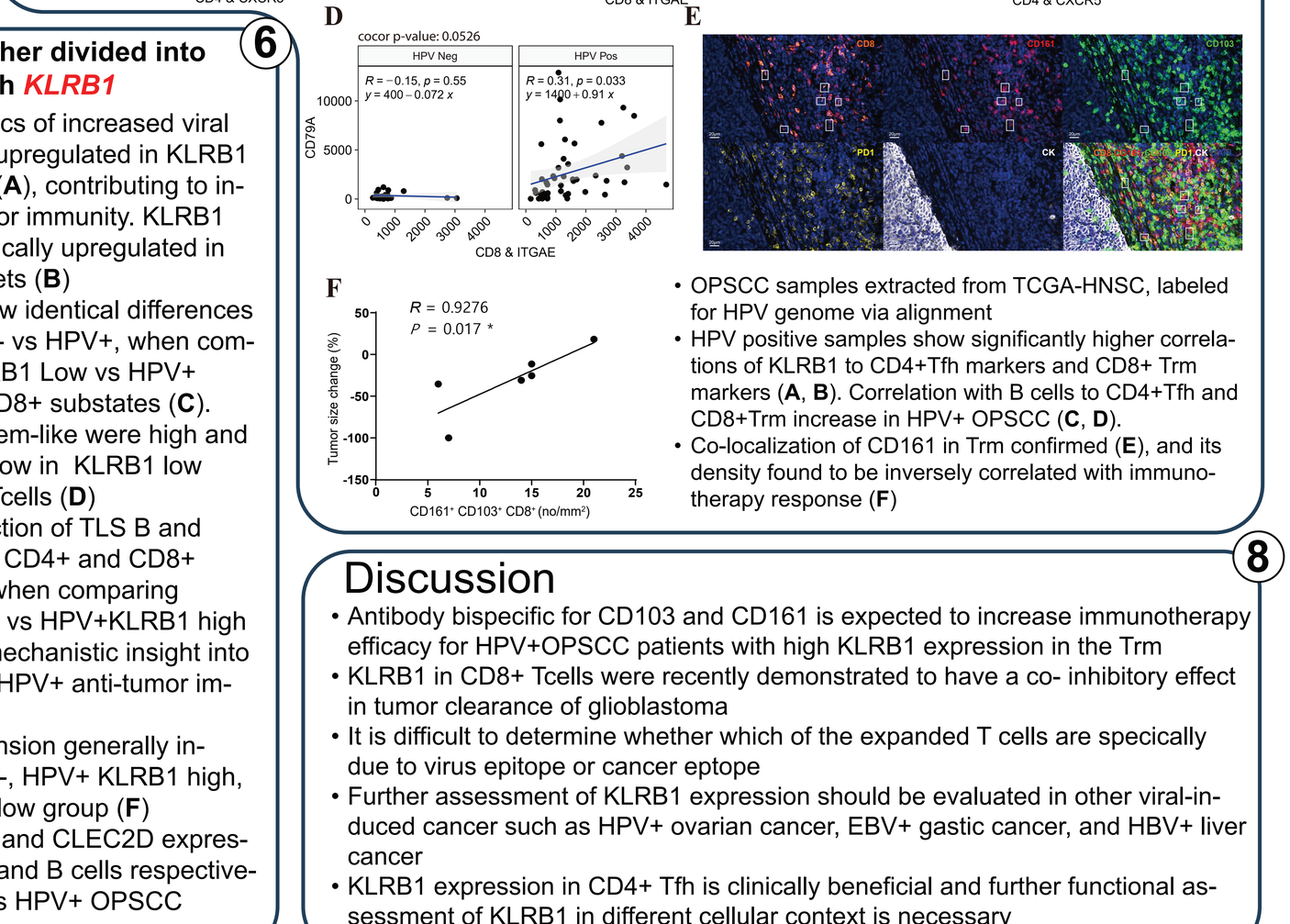
- Clonal expansion mostly oberserved in exhausted CD8+ Tcells (**E**) with clonal expansion increased in all HPV+ subclusters (**F**)
- Antigen presenting functions, and T cell functions related to anti-tumor immunity upregulated in expanded T cells of HPV+ (**G**), possibly due to additional viral activation.
- Cell-cell interactions among CD8+ Tcells, CD4+ Tcells, and B cell increased in HPV+, especially interaction of TLS B and CD20+ Bcells to T cell subsets (**H**)

HPV+ cells further divided into two groups with *KLRB1*



- Temporal dynamics of increased viral response genes upregulated in KLRB1 low CD8+ Tcells (**A**), contributing to increased anti-tumor immunity. KLRB1 was found specifically upregulated in HPV+ CD8 subsets (**B**)
- RNA velocity show identical differences observed in HPV- vs HPV+, when comparing HPV+KLRB1 Low vs HPV+ KLRB1 high in CD8+ substates (**C**). Persistence of stem-like were high and exhausted were low in KLRB1 low group for CD8+ Tcells (**D**)
- Increased interaction of TLS B and CD20+ B cells to CD4+ and CD8+ Tcells observed when comparing HPV+KLRB1 low vs HPV+KLRB1 high cells, providing mechanistic insight into heterogeneity of HPV+ anti-tumor immunity (**E**)
- TCR clonal expansion generally increase from HPV-, HPV+ KLRB1 high, to HPV+ KLRB1 low group (**F**)
- KLRB1 and its ligand CLEC2D expression in CD8 Trm and B cells respectively clearly stratifies HPV+ OPSCC patients (**G**)

Cell-cell interactions among CD8+ Tcells, CD4+ Tcells, and B cell increased in HPV+, especially interaction of TLS B and CD20+ Bcells to T cell subsets (H)



- OPSCC samples extracted from TCGA-HNSC, labeled for HPV genome via alignment
- HPV positive samples show significantly higher correlations of KLRB1 to CD4+Tfh markers and CD8+ Trm markers (**A**, **B**). Correlation with B cells to CD4+Tfh and CD8+Trm increase in HPV+ OPSCC (**C**, **D**).
- Co-localization of CD161 in Trm confirmed (**E**), and its density found to be inversely correlated with immunotherapy response (**F**)

Discussion

- Antibody bispecific for CD103 and CD161 is expected to increase immunotherapy efficacy for HPV+OPSCC patients with high KLRB1 expression in the Trm
- KLRB1 in CD8+ Tcells were recently demonstrated to have a co- inhibitory effect in tumor clearance of glioblastoma
- It is difficult to determine whether which of the expanded T cells are specically due to virus epitope or cancer eptope
- Further assessment of KLRB1 expression should be evaluated in other viral-induced cancer such as HPV+ ovarian cancer, EBV+ gastic cancer, and HBV+ liver cancer
- KLRB1 expression in CD4+ Tfh is clinically beneficial and further functional assessment of KLRB1 in different cellular context is necessary