Cytotoxic CD4+ T Cells Contribute to Anti-Tumor Immune Responses in NSCLC

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INTRODUCTION

- Direct tumor cell killing in non-small cell lung cancer (NSCLC) is thought to occur through cytotoxic CD8+ T cells, which recognize antigen presented via class I human leukocyte antigen (HLA-I).
- Downregulation of HLA-I antigen presentation facilitates immune evasion and confers an evolutionary advantage to the tumor. The rate of loss of heterozygosity at the HLA-I locus (HLA-LOH) has been reported as high as 40% in NSCLC patients and has been associated with worse survival on checkpoint inhibitor (CPI) regimens.
- However, some patients still respond to CPI, despite experiencing downregulation of HLA-I in their tumor. This suggests that a HLA-I independent pathway for anti-tumor immunity exists.
- CD4+ T cells recognize antigen via class II HLA (HLA-II) rather HLA-I, but their cytotoxic ability in NSCLC tumors remains poorly characterized.

We characterize a population of CD4+ T cells with a cytotoxic phenotype that is associated with effective anti-tumor immune responses.

METHODS

Single Cell Profiling

- Single cell profiling using the 10X Genomics Chromium platform was performed on 10 NSCLC dissociated tumor samples.
- Samples were split into CD45+ and CD45- fractions and single cell RNA sequencing was performed on both fractions. Single cell TCR and cell surface protein profiling were also performed on the CD45+ fraction.
- Raw sequencing files were processed through the 10X CellRanger pipeline and then analyzed using scanpy[1] and scirpy[2]. Scrublet[3] was used for doublet detection and BBkNN[4] was used for batch correation. HLA typing and quantification was performed using ArcasHLA[5] and scHLAcount[6].

Real World NSCLC Cohort Analysis

- We used the Tempus Labs oncology database to identify 148 de-identified records of patients with metastatic, non-squamous NSCLC who were treated with an FDA approved CPI regimen.
- Samples were profiled using targeted oncology panel sequencing or whole exome DNA sequencing, and whole transcriptome RNA sequencing on CPI naive tumor samples.
- Response to therapy was evaluated using time to progression (TTP), defined as the time from CPI start to the first progression event, censored on the last known physician encounter.

RESULTS

Figure 1: Single cell RNAseq identifies a subset of tumor infiltrating cytotoxic CD4+ T cells in NSCLC

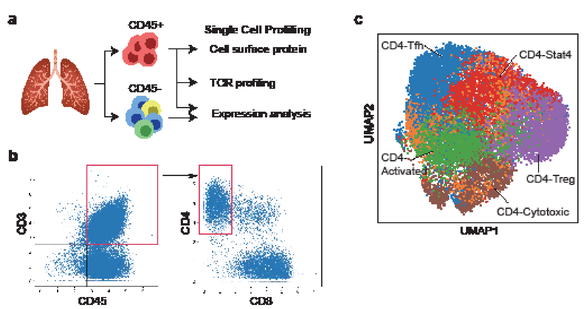

Figure 1. a) Schematic overview of the experimental design. **b)** Gating strategy for the computational isolation of the CD4+ T cell compartment. **c)** Non-negative matrix factorization (NMF) identified distinct transcriptional programs in the CD4+ T compartment. UMAP projection shows the cells labeled based on the most highly weighted transcriptional program.

Figure 2: Cytotoxic CD4+ T cells have heterogenous expression patterns of cytotoxic genes and upregulate *IFNG* and *PDCD-1*

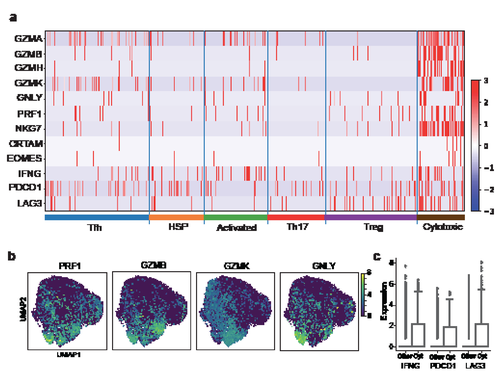

Figure 2. a) Heatmap of key cytotoxic and immune checkpoint gene expression in cytotoxic CD4+ T cell program. **b)** UMAP projections display the distinct patterns of expression for key cytotoxic genes. **c)** Expression of checkpoints, *IFNG*, *PDCD1*, and *LAG3*, is higher in the cytotoxic population compared to the other CD4+ T cells ($p<0.0001$, $p<0.0001$, $p<0.0001$, Mann Whitney U).

Figure 3: Cytotoxic CD4+ T cells are clonally expanded in NSCLC

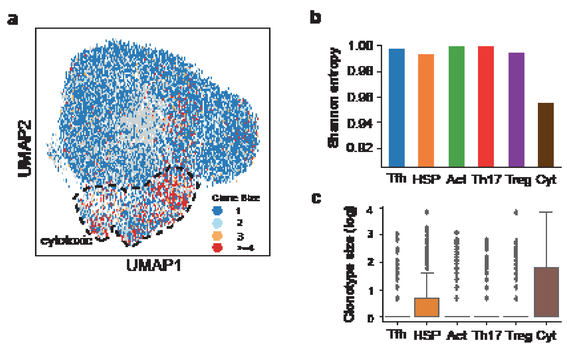

Figure 3. a) UMAP projection showing the clone size associated with the TCR for each cell. **b)** TCR diversity, as measured by Shannon entropy, for each NMF cluster. **c)** Log transformed TCR clonotype size of the cytotoxic CD4+ T cell population compared to other CD4+ T cells ($p<0.001$, Kruskal Wallis).

Figure 4: A subpopulation of tumor cells express HLA-II in NSCLC, allowing for direct antigen presentation to CD4+ T cells

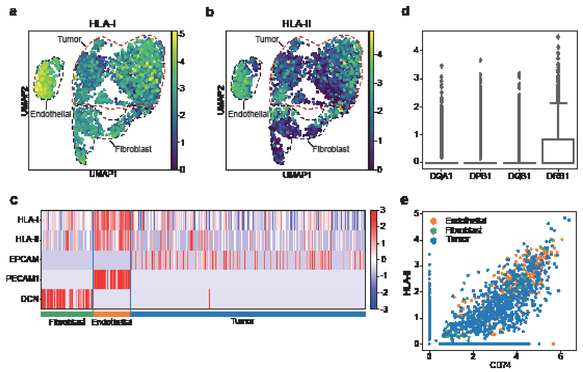

Figure 4. UMAP projections show the expression of **a)** HLA-I and **b)** HLA-II in the CD45- fraction. **c)** Heatmap shows the expression of HLA-I, HLA-II and key lineage markers. **d)** Boxplots show the log transformed expression of the individual HLA-II genes assessed ($p<0.0001$, Kruskal-Wallis). **e)** Expression of HLA-II and its' chaperone, CD74 (invariant chain) is highly correlated ($R=0.627$, $p<0.0001$, Pearson correlation).

Figure 5: Gene signature of cytotoxicity is associated with CPI response in a real world NSCLC cohort, irrespective of HLA-I status

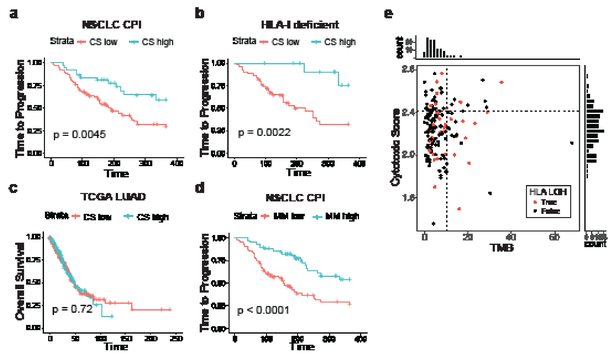

Figure 5. We developed a 20 gene signature for cytotoxicity. KM plots show that the cytotoxic score (CS) is significantly associated with TTP in the **a)** Tempus NSCLC CPI cohort (HR=0.42), including patients in the **b)** HLA-I deficient (LOH, homozygous or B2M mutation) sub-cohort (HR=0.16). **c)** CS is not associated with survival in the TCGA lung adenocarcinoma (LUAD) cohort (HR=0.99), which was primarily treated with chemotherapy. **d)** Combining CS with TMB into a simple multimodal model (MM) improves CPI response prediction compared to either biomarker alone (HR=0.37). **e)** TMB and CS are not significantly correlated ($R=0.031$, $p=0.71$, Pearson correlation).

CONCLUSIONS

- Cytotoxic CD4+ T cells are present in the tumor infiltrating immune compartment in NSCLC patients.
- Cytotoxic CD4+ T cells upregulate immune checkpoint genes and are clonally expanded.
- NSCLC tumor cells can present antigen directly to cytotoxic CD4+ T cells via HLA-II.
- An RNA signature of cytotoxicity is associated with CPI response in NSCLC, independent of HLA-I status.

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