Exclusion of CD8 T Cells from Tumor Epithelium is Associated with Worse Immune Checkpoint Blockade (ICB) Therapy Response and Survival in Non-Small Cell Lung Cancer (NSCLC)

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Background

Tumor-infiltrating lymphocytes (TILs) are crucial for patient response to ICB treatment and prognosis [1]. However, tumors characterized by immune exclusion, where T cells are concentrated in the tumor-associated stroma but sparsely present or absent in the tumor epithelium, may not respond well to ICB therapy [2]. Discoidin Domain Receptor 1 (DDR1), a tumor expressing collagen-binding receptor tyrosine kinase, has been implicated in cancer invasion, progression, and immune exclusion [3]. This study aims to utilize multiplex immunofluorescence (mIF) staining of NSCLC tumors to elucidate the impact of immune exclusion on ICB response and prognosis, and to evaluate TIL location in the tumor microenvironment (TME) and associated gene signatures as potential biomarkers of response, and DDR1 as a potential therapeutic target.

Methods

Tumors from 100 NSCLC patients enrolled in the Institut Bergonié profiling precision medicine study (NCT02534649) were evaluated by RNA-seq and mIF staining with a panel containing PanCK, COL1A1, CD8, and DDR1, followed by image analysis (Fig. 1 A, B). Patients were split evenly between Lung Adenocarcinoma (LUAD) and Lung Squamous Cell Carcinoma (LUSC), and between responders and non-responders to ICB therapy. A continuous Immune Exclusion Score (IES) was computed based on CD8 T cell densities in the tumor epithelium and tumor stroma (Fig. 1 C). CD8 T cell density (whole tumor, tumor epithelium, and tumor stroma) and IES were compared in responders vs non-responders and evaluated against survival to identify potential biomarkers of ICB response. Gene signatures associated with IES were identified and tested in an independent lung cancer cohort with ICB outcome data [4].

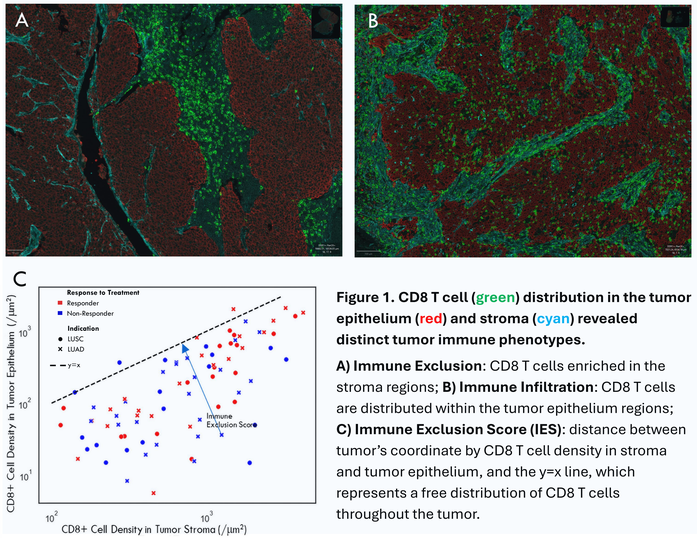

Figure 1. CD8 T cell (green) distribution in the tumor epithelium (red) and stroma (cyan) revealed distinct tumor immune phenotypes.

A) Immune Exclusion: CD8 T cells enriched in the stroma regions; **B) Immune Infiltration**: CD8 T cells are distributed within the tumor epithelium regions; **C) Immune Exclusion Score (IES)**: distance between tumor's coordinate by CD8 T cell density in stroma and tumor epithelium, and the y=x line, which represents a free distribution of CD8 T cells throughout the tumor.

Results

Higher Tumor Epithelium CD8 T cell Density In Responders to ICB

CD8 T cell density in the tumor epithelium was higher in responders than non-responders, while no difference in CD8 T cell density in tumor stroma was observed.

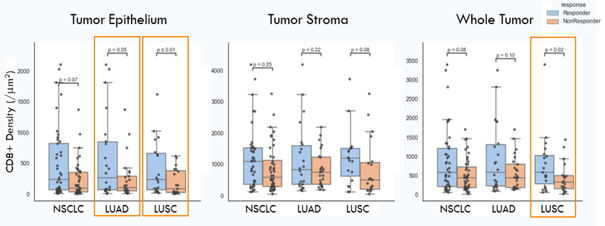

Figure 2. CD8 T cell densities in different tumor regions in NSCLC patients responding or not responding to ICB treatment

This figure compares CD8 T cell densities in the tumor epithelium, stroma, and whole tumor between NSCLC patients who respond and those who do not respond to ICB treatment. The NSCLC cohort includes patients from both LUAD and LUSC subtypes.

CD8 T Cell in Tumor Epithelium, not Stroma, Makes Difference in Patient Survival

Patients with high CD8 T cell density in tumor epithelium, but not in tumor stroma, exhibited improved overall and progression-free survival.

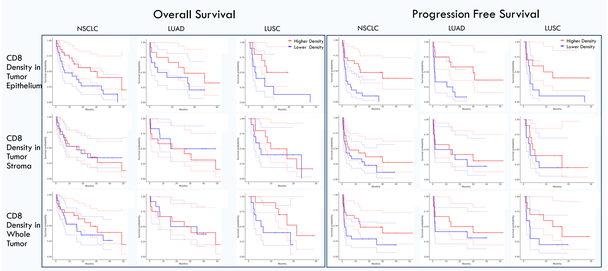

Figure 3. Differences in overall survival (OS) and progression-free survival (PFS) in NSCLC patients with high or low CD8 cell densities in tumor epithelium, tumor stroma, and whole tumor

This figure compares OS and PFS between patients with higher CD8 T cell density (top 25%, red) and lower CD8 T cell density (bottom 25%, blue). Higher CD8 T cell density in the tumor epithelium is associated with the greatest survival benefit, while higher CD8 T cell density in the tumor stroma shows very small or no survival benefit. The survival benefit is modest for higher CD8 T cell density in the whole tumor. Dotted lines indicate the 95% confidence intervals.

Immune Exclusion Score (IES) and Patient Overall Survival

Patients with high IES had shorter survival than patients with low IES, independent of PD-L1 expression.

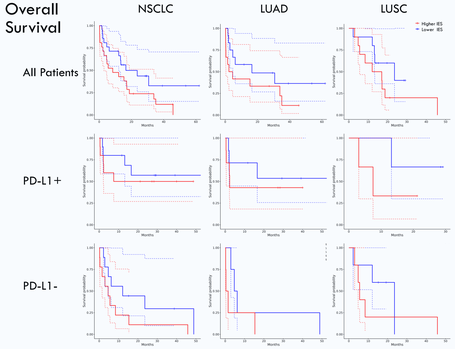

Figure 4. Differences in overall survival (OS) in NSCLC patients with high or low Immune Exclusion Score (IES)

Comparison between higher IES (top 25%, red) and lower IES (bottom 25%, blue) patients. Tumors with high IES exhibit shorter OS and PFS in LUAD, LUSC, and combined cohorts, independent of PD-L1 expression. .Dotted lines indicate the 95% confidence intervals

Independent Validation of Association between CD8 T Cell Exclusion and ICB Response

Expression of the CD8 T cell exclusion signature was higher in ICB non-responders from an independent lung cancer study [4].

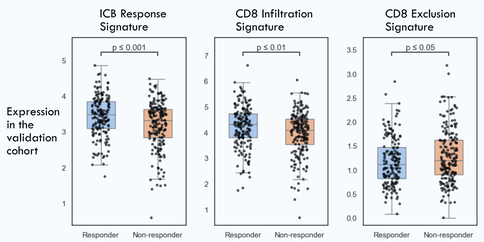

Figure 5. Similar trend of expression of ICB response and CD8 infiltration/exclusion gene signatures in an independent cohort

Gene signatures were extracted from the RNA-seq data of our NSCLC cohort. Their expression, when examined in an independent validation cohort of 355 lung cancer ICB-treated clinical samples [4], exhibited a similar trend to that observed in our NSCLC cohort.

Indication Specific Association of Drug Target Gene Expression and Immune Exclusion

DDR1 gene and signature expression were correlated with immune exclusion in LUAD, while DDR1+ cell density (protein expression) was correlated with immune exclusion in LUSC.

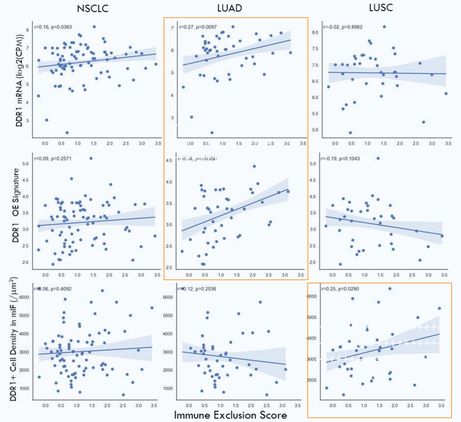

Figure 6. Indication specific DDR1 expression correlation with tumor immune exclusion scores

DDR1 mRNA expression and the DDR1 Overexpression signature (Yang et al., 2022) are strongly correlated with the CD8 T cell-based Immune Exclusion Score in LUAD. DDR1+ cell density in mIF slides is also correlated with the Immune Exclusion Score in LUSC.

Conclusions

CD8 T cell density in the tumor epithelium, rather than the tumor stroma, is associated with response and survival in ICB-treated NSCLC patients. Additionally, CD8 T cell exclusion from the tumor epithelium impedes patient response and reduces survival. The spatial distribution of CD8 T cells within the tumor and immune exclusion-associated gene signatures could serve as potential biomarkers for ICB treatment in NSCLC. DDR1 is a potential therapeutic target to address immune exclusion.

References

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2. Clifton, G.T., et al. Developing a definition of immune exclusion in cancer: results of a modified Delphi workshop. J Immunother Cancer. 2023; 11.
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