Large-scale transcriptomic profiling of the tumor immune microenvironment in ALK+ lung cancer

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Introduction

Anaplastic Lymphoma Kinase (ALK) re-arrangements define a distinct molecular subset of non-small cell lung cancer (NSCLC) that predominantly affects younger patients and those with sparse or no smoking exposure. These patients do not derive significant clinical benefit from currently available immune checkpoint inhibitors. Elucidating the mechanisms underlying the immunosuppressive tumor microenvironment will help inform the development of novel immunotherapy approaches for ALK+ NSCLC.

Objectives

Characterize major immune components of the tumor microenvironment (TME) by comprehensive transcriptomic and immunohistochemistry (IHC) analyses

Materials and Methods

- We analyzed NGS data from 5490 NSCLC patients that underwent DNA (592 Gene Panel, NextSeq, or WES, NovaSeq) and RNA (NovaSeq, WTS) sequencing at Caris Life Sciences (Phoenix, AZ).
- 374 ALK-rearranged cases were evaluated, along with 3169 KRAS-mut (STK11/KEAP1-wt) and 1947 EGFR-mut cases serving as comparators with known heterogenous and inert immune TMEs, respectively.
- PD-L1 (22C3) was evaluated by IHC. Immune cell fractions were inferred using quanTseq (Finotino, 2019).
- Gene expression profiles were analyzed for a T cell-inflamed signature (TIS, Cristescu 2018) predictive of response to immunotherapy and for other immune modulatory genes such as IFNG, GZMB, TGFB1, and those of the adenosine pathway (CD73/NTSE, CD39/ENTPD1, ADORA1, ADORA2A/B). A significant difference between genomic subgroups was defined as fold-change > 1.2.
- In an independent cohort of 13 ALK+ NSCLC and 5 KRAS+ NSCLC cases, density and spatial organization of CD4+ and CD8+ T cells, Tregs, major myeloid lineage cells, PD-L1, and CD73 were assessed by quantitative IHC (Vectra Polaris [Akoya Biosciences] and HALO [Indica Labs])

Results

Figure 1: TMB and PD-L1 Prevalence

Median TMB (mut/MB) was 3.0, 0.9, and 4.0 for ALK, KRAS, and EGFR, respectively. PD-L1 TPS > 1 and TPS > 50.

Table 1: Immune checkpoinst, CD73/adenosine

LAG-3 (fold-change -1.4 p<0.001), CD73/NTSE (fold-change -1.7 p<0.001), and ADORA2A (fold-change -1.4, p<0.001) were decreased while ADORA1 (fold-change 1.3, p<0.001) was increased compared to KRAS-mut.

Figure 2: Immune subsets and activation markers

Immune subset cell fractions (a) and immune-related genes (c) with fold-change > 1.2 (compared to KRAS-mut) named in red. Box plots with fold-change comparison p-values for (b) immune subsets and (d) genes.

Figure 3: Oncoprint sorted by T cell-inflamed (TIS) score

Each column represents a tumor sample. This oncoprint summarizes quantitative immune cell subsets, PD-L1, TMB. No association between ALK+ tumors and TIS.

Figure 4: Dual-color quantitative IHC

(a) ALK+ dual-stained IHC example, showing paucity of CD8+ cells, including in the presence of high PD-L1. In panel (b), top row is ALK+ sample with significant FoxP3, CD4, CD11b (MDSC) staining. Bottom row is KRAS-mut. (c) Comparison of cell density in limited IHC cohort. CD11c+ dendritic cells, CD162+ M2 Mφ

Conclusion

Despite high levels of PD-L1, ALK+ tumors exhibit multiple features of an inert immune TME, primarily characterized by low TMB and decreased CD8+ T cells and immune activation markers. While immunosuppressive factors such as M2 macrophages and adenosine signaling may be targeted, strategies to enhance immunogenicity will be critical for an effective immune response in ALK+ NSCLC.