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 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 quanTIseq (Finotello, 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/NT5E, 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, PDL1, and CD73 were assessed by quantitative IHC (Vectra Polaris [Akoya Biosciences] and HALO [Indica Labs])

Results

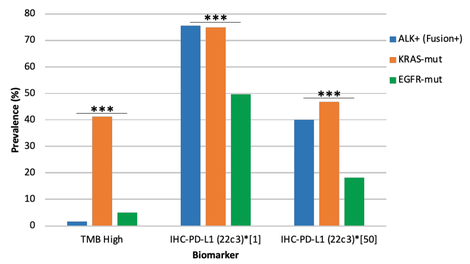
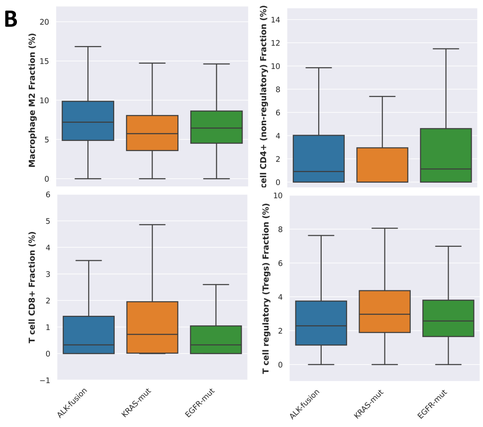


Figure 1: TMB and PD-L1 Prevalence
Median TMB (mut/MB) was 3.0, 9.0, and 4.0 for ALK, KRAS, and EGFR, respectively. PD-L1 TPS > 1 and TPS > 50.

A

	Median Cell Fraction		
	ALK+ (Fusion+)	KRAS-mut	EGFR-mut
B cell	4.3%	4.4%	4.3%
Macrophage M1	6.0%	6.9%	5.9%
Macrophage M2	7.2%	5.7%	6.5%
Monocyte	0.0%	0.0%	0.0%
Myeloid dendritic cell	0.0%	0.0%	0.0%
NK cell	2.7%	2.6%	3.4%
Neutrophil	6.9%	6.3%	7.2%
T cell CD4+ (non-regulatory)	0.9%	0.0%	1.1%
T cell CD8+	0.3%	0.7%	0.3%
T cell regulatory (Tregs)	2.3%	3.0%	2.6%

B



	Median Expression (TPM)		
	ALK+ (Fusion+)	KRAS-mut	EGFR-mut
CTLA4	3.08	3.64	3.04
HAVCR2 (TIGIT)	32.08	30.26	28.39
C10orf54 (VISTA)	20.26	18.72	17.71
LAG3	1.15	1.62	1.06
ADORA1	11.851	9.419	9.591
ADORA2A	1.411	2.029	1.732
ADORA2B	6.612	7.135	6.253
NT5E (CD73)	65.000	107.495	62.399
CD38	6.687	7.764	7.185
ENTPD1 (CD39)	21.248	21.882	20.897

Table 1: Immune checkpoints, CD73/adenosine
LAG-3 (fold-change -1.4 p<0.001), *CD73/NT53* (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.

C

	Median Expression (TPM)		
	ALK+ (Fusion+)	KRAS-mut	EGFR-mut
IFNG	0.918	1.374	0.830
GZMB	7.478	11.733	5.529
FOXP3	3.414	3.899	3.328
TGFB1	36.502	45.696	39.447
CD274 (PD-L1)	11.376	17.760	9.161

D

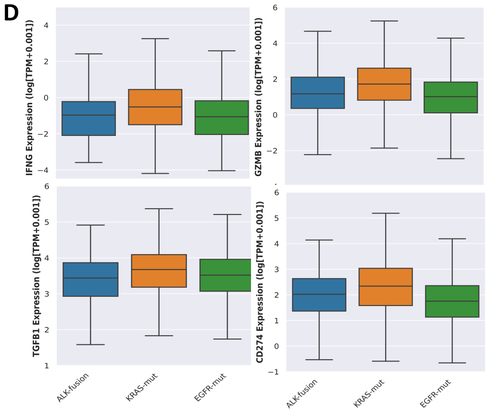


Figure 2: Immune subsets and activation markers
Immune subset cell fractions **(a)** and immune-related genes **(c)** with fold-change > 1.2 (compared to *KRAS*-mut) named in red. Box plots with fold-change comparison p-values for **(b)** immune subsets and **(d)** genes.

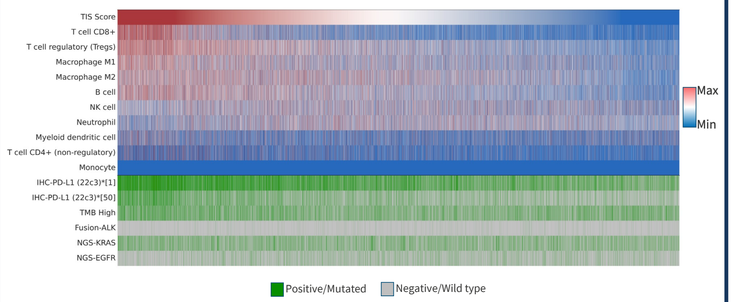
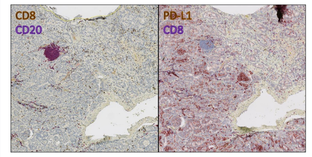


Figure 3: Oncoprint sorted by T cell-inflamed (TIS) score
Each column represents a tumor sample. This oncoprint summarizes quantiSeq immune cell subsets, PD-L1, TMB. No association between ALK+ tumors and TIS.

A



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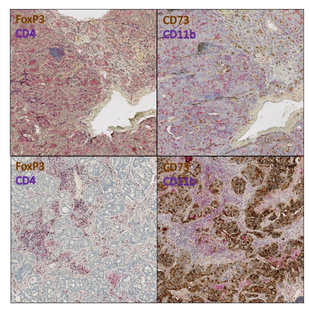

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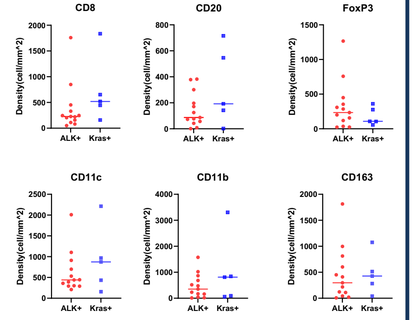

Figure 4: Dual-color quantitative IHC
(a) *ALK*+ dual-stained IHC example, showing paucity of CD8+ cells, including in the presence of high PD-L1. In panel **(b)**, top row is *ALK*+ sample with significant FoxP3, CD4, CD11b (MDSC) staining. Bottom row is *KRAS*-mut. **(c)** Comparison of cell density 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.

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