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# Drug-resistant subpopulation in *ALK*-rearranged lung adenocarcinoma release CCL20 to modulate tumor immune microenvironment

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## Abstract

Acquired resistance to tyrosine kinase inhibitor (TKIs) is the major therapeutic obstacle to maintenance treatment of advanced-stage lung adenocarcinomas. Significant subclonal transcriptomic variation results in diverse intratumoral heterogeneity that may contribute to emerge of drug resistance. Immune checkpoint inhibitors (ICIs) can confer a durable therapeutic response for advanced-stage lung cancer without oncogenic driver mutations. However, lung cancer bearing driver mutations appear much less sensitive to ICIs. In this study, we explored the intratumoral heterogeneity of TKI-resistant anaplastic lymphoma kinase (ALK)-rearranged lung adenocarcinoma organoids using single-cell RNA-sequencing (scRNA-seq) transcriptomic analysis. We have identified that chemokine ligand 20 (CCL20) was highly expressed in a subpopulation of ALK-TKI-resistant cells. CCL20 can recruit T-helper 17 (Th17) lymphocytes into the tumor creating an immunosuppressive microenvironment favored tumor progression. These persistent cancer cells marked with high surface intracellular adhesion molecule 1 (ICAM-1) level were more resistant against ALK-TKI treatment. These subpopulations of cells demonstrated stronger in vitro tumorigenicity and expressed a higher level of stem cell transcription factors. Moreover, ICAM-1$^{high}$ ALK-TKI-resistant cells released more CCL20 and induce migration of Th17. In conclusion, our findings demonstrated that subpopulation of persistent ALK-TKI-resistant lung adenocarcinoma cells can release CCL20 to modulate the tumor microenvironment which may also contribute to insensitivity to ICIs. These results shed new lights on the possibility of bridging ICI with CCL20 neutralization in lung adenocarcinoma bearing driver mutations.

## Introduction

Tyrosine kinase inhibitors (TKIs) including crizotinib or alectinib, that target *ALK*-rearrangement are currently first-line treatment for advanced non-small cell lung cancer (NSCLC). Intratumoral heterogeneity and clonal evolution could fuel therapeutic resistance of tumor. Although improving efficacy over generations of TKIs has been demonstrated, NSCLC patients treated with TKIs commonly progress within one to two years due to the development of acquired resistance. Intratumoral heterogeneity accounts for cancer progression and resistance to TKI either due to differential sensitivity of subclones or acquired mutations, leading to incomplete eradication of tumor cells by TKI treatment, and ultimately relapse of lung cancer. Immune checkpoint inhibitors (ICIs) demonstrated durable treatment response but not in NSCLC patients with driver mutations, further investigations on the tumor immune microenvironment are required. In this study, we hypothesized that drug-tolerant persistent cancer cells are presence in heterogenous subpopulation of tumor and confer development of drug resistance to TKI treatment. scRNA-seq was applied to resolve the intratumoral heterogeneity of *ALK*-rearranged lung adenocarcinoma organoids with acquired resistance and identified distinct markers were identified these may be prognostic and therapeutic targets.

## Methodology

Single-cell RNA sequencing analysis was performed on two established *ALK*-rearranged NSCLC tumor organoids together with their TKI resistant clones. Subsequent functional studies including flow cytometry, qPCR, ELISA and *in silico* experiments were performed.

## Discussion and Conclusion

This is the first study demonstrating that subpopulation of persistent ALK-TKI-resistant lung adenocarcinoma cells can release CCL20 to modulate the tumor microenvironment, and this may also contribute to insensitivity to various drug treatment including TKI or ICI.

**Reference**

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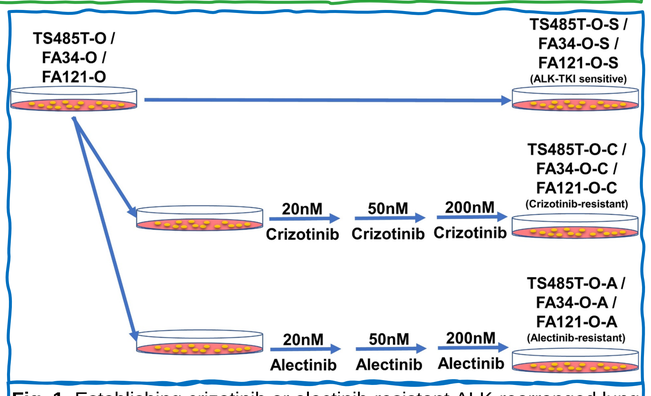
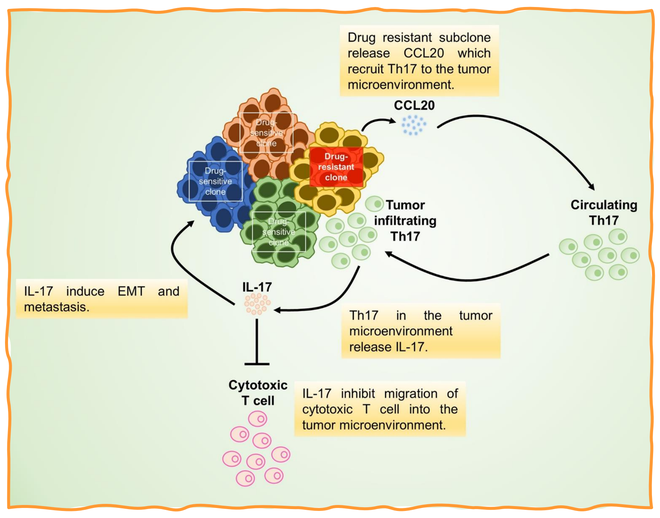



**Fig. 1.** Establishing crizotinib or alectinib-resistant ALK-rearranged lung adenocarcinoma tumor organoid.




**Hypothetical model showing the role of CCL20 and its downstream mechanism in modulating the tumor immune microenvironment.** Subpopulation of drug resistant tumor cells release CCL20 to recruit Th17 into the tumor microenvironment. The Th17 in the tumor bed release IL-17 which on one hand prevent the infiltration of CD8$^{+}$ cytotoxic T cells, the IL-17 could also induce EMT and metastasis of tumor cells on the other hand.

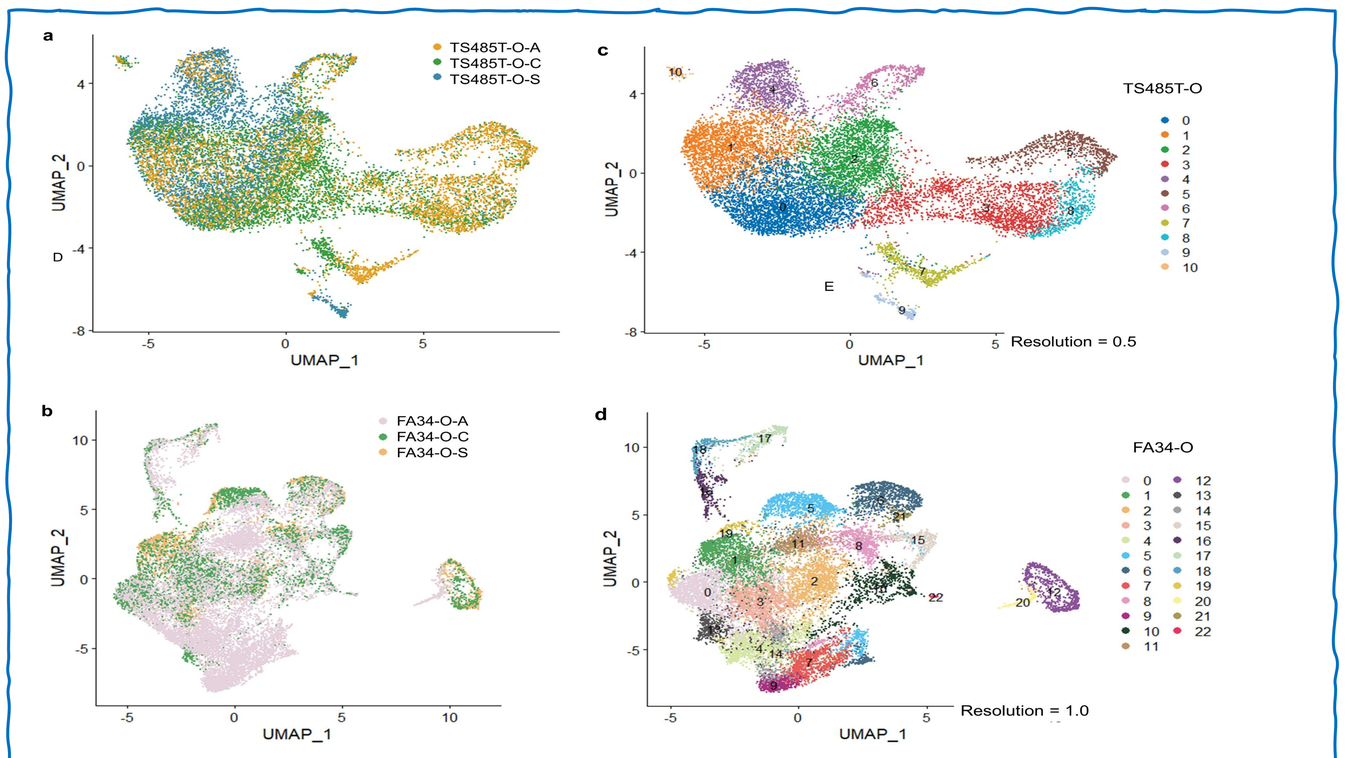



**Fig. 2.** UMAP representation of TS485T-O sensitive or resistant cells colored by treatment (**a** and **b**) or by clusters (**c** and **d**).

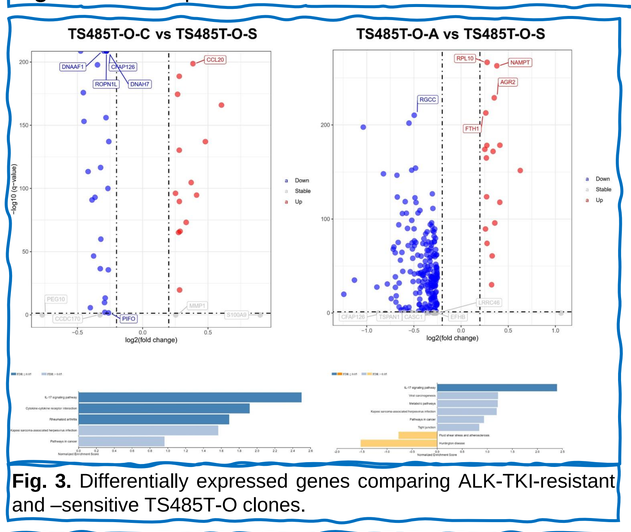



**Fig. 3.** Differentially expressed genes comparing ALK-TKI-resistant and –sensitive TS485T-O clones.

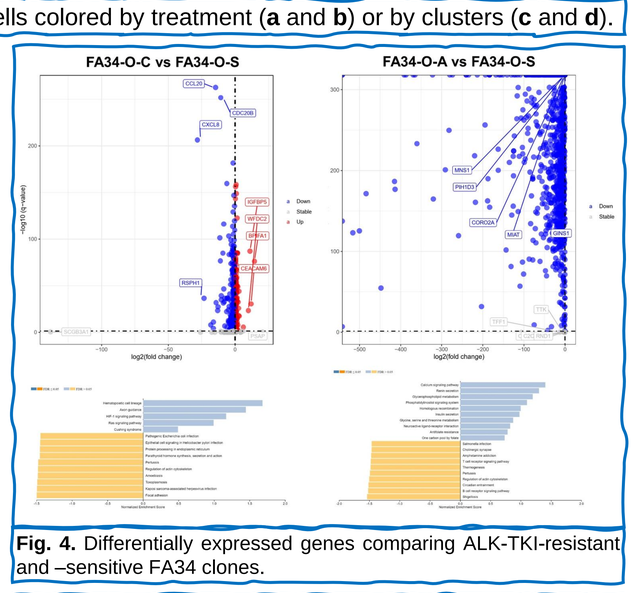



**Fig. 4.** Differentially expressed genes comparing ALK-TKI-resistant and –sensitive FA34 clones.

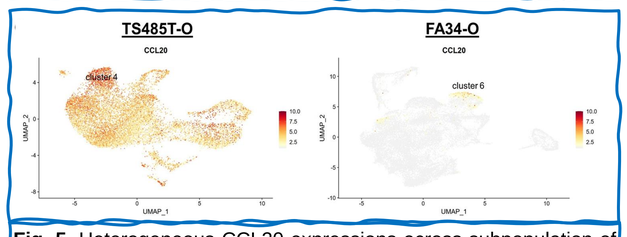



**Fig. 5.** Heterogeneous CCL20 expressions across subpopulation of cells.

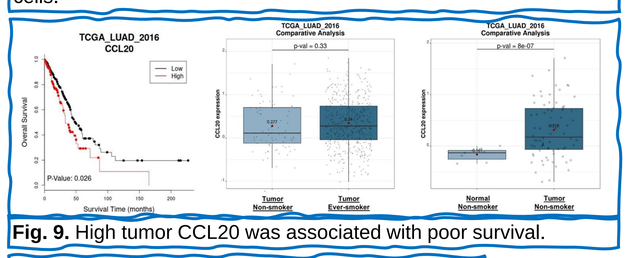



**Fig. 9.** High tumor CCL20 was associated with poor survival.

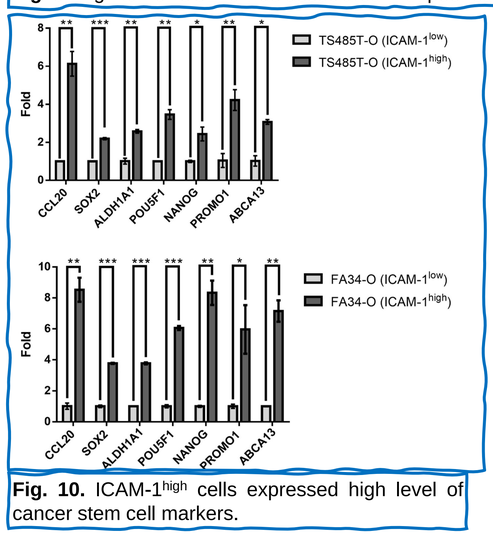



**Fig. 10.** ICAM-1$^{high}$ cells expressed high level of cancer stem cell markers.

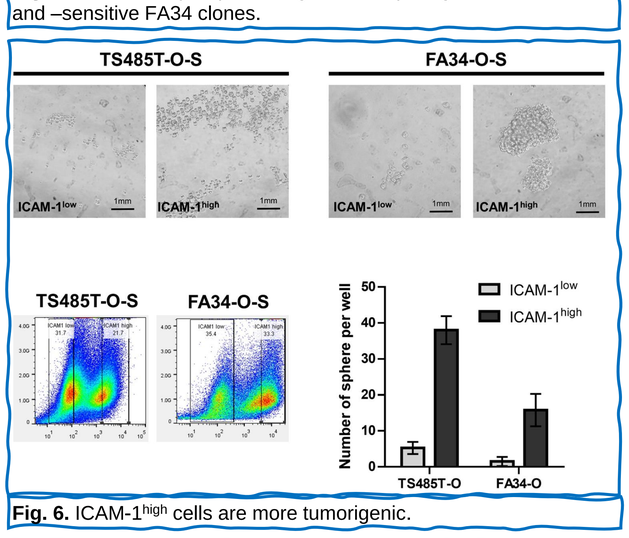



**Fig. 6.** ICAM-1$^{high}$ cells are more tumorigenic.

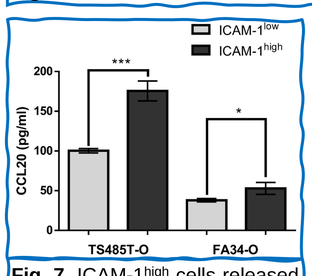



**Fig. 7.** ICAM-1$^{high}$ cells released more CCL20.

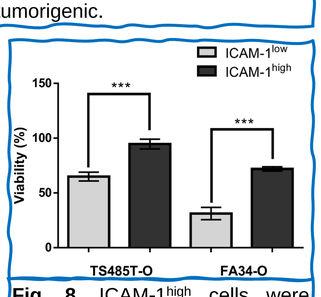



**Fig. 8.** ICAM-1$^{high}$ cells were more resistant against crizotinib treatment.

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