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REVIEW

Characterizing the immune tumor microenvironment in ALK fusion-positive lung cancer: state-of-the-art and therapeutical implications

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ABSTRACT

Introduction: Approximately 5% of non-small cell lung cancer (NSCLC), exhibits anaplastic lymphoma kinase (ALK) rearrangements. EML4-ALK fusions account for over 90% of ALK rearrangements in NSCLC. The advent of treatment targeting ALK has significantly improved survival rates in patients with advanced ALK-positive NSCLC. However, the emergence of resistance mechanisms and the subsequent progression disease inevitably occurs. The tumor immune microenvironment (TIME) plays a pivotal role in lung cancer, influencing disease development, patient's outcomes, and response to treatments.

Areas covered: The aim of this review is to provide a comprehensive characterization of the TIME in ALK rearranged NSCLC and its intrinsic plasticity under treatment pressure.

Expert opinion: Recognizing the fundamental role of the TIME in cancer progression has shifted the paradigm from a tumor cell-centric perspective to the understanding of a complex tumor ecosystem. Understanding the intricate dynamics of the TIME, its influence on treatment response, and the potential of immunotherapy in patients with ALK-positive NSCLC are currently among the primary research objectives in this patient population.

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1. Introduction

Approximately 5% of non-small cell lung cancer (NSCLC), especially adenocarcinoma, exhibits ALK rearrangements [1]. EML4-ALK fusions account for over 90% of ALK rearrangements in NSCLC [2]. This rearrangement is due to a specific inversion [inv(2)(p21p23)], juxtaposing the N-terminal of the EML4 gene promoter with the kinase domain of the ALK gene [3,4]. The fusion of EML4 with ALK promotes ligand independent ALK activation and constitutive kinase activity, fostering cancer cell proliferation and survival [5].

The advent of tyrosine kinase inhibitors (TKIs) targeting ALK has significantly improved survival rates in patients with advanced ALK-positive NSCLC [6]. Currently, first (crizotinib), second (alectinib, and brigatinib), and third generation (lorlatinib) ALK TKIs are available. Second and third generation TKIs are nowadays preferred as first-line therapy due to their superior progression-free survival (PFS) and overall response rate (ORR) compared to crizotinib [7–10]. Notably, brigatinib, alectinib, and lorlatinib demonstrate considerable intracranial response rates [7–9].

However, the emergence of resistance mechanisms and the subsequent disease progression inevitably occurs [11]. The ability of cancer cells to exhibit biological plasticity leads to a prompt adaptation to treatment, thereby limiting the effectiveness of precision approaches in cancer treatment [12,13]. Of note, the tumor immune microenvironment (TIME) plays a pivotal role in lung cancer, influencing disease development, patient's outcomes, and response to treatments [14]. TIME affects responses to immune checkpoint inhibitors and TKIs and, conversely, these therapies modulate the composition of the TIME itself [14–16].

Recognizing the fundamental role of the TIME in cancer progression has shifted the paradigm from a tumor cell-centric perspective to the understanding of a complex tumor ecosystem. In this light, the aim of this review is to provide a comprehensive characterization of the tumor microenvironment in ALK rearranged NSCLC and its intrinsic plasticity under treatment pressure.

2. Immune tumor microenvironment composition

Besides tumor cells, TIME includes immune cells, fibroblasts, pericytes, adipocytes, endothelial cells, carcinoma-associated fibroblasts (CAFs) [17]. Of note, TIME is also composed by

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Article highlights

- Patients with ALK translocated disease have peculiar TIME features, i.e. a reduced functionality of effector T cells and an increased expression of PD-1, LAG-3, and TIM-3.
- ALK positive NSCLC that develop resistance to ALK TKIs show a low presence of TCD8+ and high presence of Treg in TIME. In NSCLC responsive to ALK TKIs, an increase in TCD8+ CD3+ cells, natural killer and gamma delta cells was observed.
- Modified IL-2, CAR-T cell therapies, and the combination of novel immune agents with anti-ALK TKIs are examples of treatments that are currently under investigation.

blood and lymph vessels, extracellular matrix (ECM), microvesicles and various cytokines and chemokines [17,18]. Globally, the components of TIME can be divided into immunogenic, such as cytotoxic CD8 T cells, Natural Killers (NK) cells, dendritic cells (DCs) and M1 tumor-associated macrophages [18], and immunosuppressive, such as Treg cells, myeloid-derived suppressor cells (MDSCs), CAFs and M2 tumor-associated macrophages [18].

Both intertumoral (i.e. sharing features that recur in patients with the same tumor type) and intratumor heterogeneity, that can be temporal (i.e. referring to changes in TIME composition occurring in the same individual over time) or spatial (i.e. different distribution of immune cells within the same tumor sample) [19], characterize TIME. Interestingly, TIME in NSCLC is subject to the influence of driver mutations, which can determine variations in tumor-infiltrating cells, immunomodulatory molecules, cytokines, and chemokines [20]. For example, EGFR mutations and MET amplifications have been associated with a poorly responsive immune environment, whereas among patients with KRAS mutation, those with co-mutation of STK11 or KEAP1 present an immunosuppressive microenvironment, while those with TP53 co-mutation are associated with greater immunogenicity [21].

3. Immune tumor microenvironment in ALK fusion-positive lung cancer

Several available data have consistently pointed toward the existence of an immunosuppressive TIME within ALK-translocated NSCLC which may involve various mechanisms, such as the recruitment of immunosuppressive cell populations, dysregulation of cytokine signaling pathways and expression of inhibitory immune checkpoints [21–23] (Figure 1).

Usually, enhanced PDL1 expression on the tumor is correlated with better responses to PD1 axis blockade [24,25]. Despite ALK-rearranged NSCLC often shows high levels of PD-L1 expression [26,27], responses to immune checkpoint inhibitors (ICIs) in these tumors have been disappointing [28]. High expression of PD-L1 in ALK-translocated NSCLC, reflecting a constitutive expression through oncogenic signaling rather than a response induced by T-cell activity, appears to be associated either with ALK-induced upregulation of HIF-1alpha and STAT3 or with the activation of downstream PI3K-AKT and MEK-ERK signaling pathways [27,29]. While studies examining different immune cell populations within TIME report heterogeneous results, together, they suggest reduced functionality of effector T cells in ALK-positive tumors compared to non-oncogene-addicted diseases [29,30].

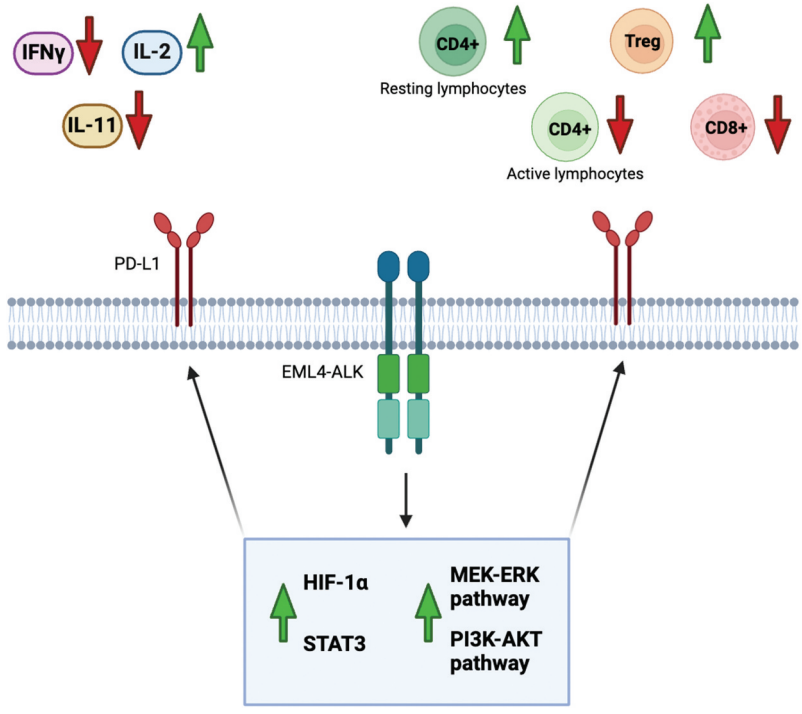

Figure 1. Tumor immunological microenvironment composition in ALK rearranged NSCLC.

The available data suggest reduced functionality of effector T cells in patients with ALK translocation disease, with decreased production of interferon gamma, low levels of granzyme B by CD8+ T lymphocytes and increased expression of PD-1, LAG-3, and TIM-3.

In patients with ALK-translocated NSCLC, an increase in resting memory CD4+ T cells and a decrease in active memory cells was also observed.

Legend: IFN, interferon; IL, interleukin; HIF, hypoxia-inducible factor.

Multispectral imaging in NSCLC EML4-ALK transgenic treatment-naïve mice detected scarcity of T cell infiltrate [22]. Gene set enrichment analysis conducted on human NSCLC with ALK rearrangement revealed, in comparison to NSCLC with wild-type (wt) ALK/RAS/EGFR, a diminished expression of genes associated with T-cell infiltration and a significant reduction in the expression of TCR-related molecules including TCRb, CD3d, CD3g, CD3z, and Lck. Furthermore, ALK positive NSCLC demonstrated a decrease of T-cell co-stimulatory molecules such as ICOS and CD28, as well as a reduction in CD80 and CTLA-4 levels [30].

To evaluate the immune biomarkers in TIME and their prognostic value in ALK-rearranged NSCLC, Zangh et al. analyzed tumor samples from 39 ALK-rearranged NSCLC patients and compared them to 40 EGFR mutant and 30 KRAS mutant patients [31]. Interestingly, ALK positive NSCLC exhibited significantly lower expression levels of CTLA4, LAG3, and TIGIT in TIME compared to EGFR mutant lung cancer ($p < 0.05$). Conversely, TIM3 expression was significantly higher in patients with ALK-positive NSCLC than in those with KRAS-positive NSCLC ($p < 0.05$). Moreover, compared to KRAS mutant NSCLC, a reduction of activated immune populations (CD3+, CD8+, Granzyme B+, and CD20+), alongside an increased expression of TIM3 was noticed in ALK-mutated NSCLC. Of note, high expression of PD-L1 and CTLA4 was linked to worse overall survival in patients treated with ALK TKIs [31]. In contrast with these results, another study, involving patients treated with alectinib, demonstrated no statistically significant associations between PD-L1 positivity and objective response rate ($p = 0.274$) or PFS (HR 0.98, 95% CI 0.37–2.61, $p = 0.97$) [32] (Table 1).

Interestingly, the CD8+ expression in tumor-infiltrating lymphocytes (TILs) may also vary depending on the treatment with ALK TKIs. A study demonstrated that while CD8+ T cell immunohistochemical positivity was observable in the majority of samples of treatment-naïve ALK-translocated NSCLC patients, infiltration of CD8+ T cells was minimal or absent in 10 out of the 13 patients receiving ALK inhibitors [33].

Furthermore, Voena et al. characterized the immune infiltrate in mouse models of NSCLC with ALK translocation. Compared to NSCLC models with wt ALK, a similar proportion of T lymphocytes, B cells, NK cells, and granulocytes was reported. However, in mice with EML4-ALK translocation, CD4+T and CD8+ T lymphocytes exhibited elevated PD-1 expression, and CD3+ and PD-1+ T cells demonstrated heightened levels of T-cell inhibitory molecules, such as LAG-3 and TIM-3. Additionally, ALK translocation mouse models showed an increase in FOXP3+ Treg cells over time [30]. In a retrospective analysis of 31 patients with ALK-rearranged NSCLC and 43 patients with wt ALK and EGFR, a higher percentage of FOXP3-positive cells, as assessed by immunohistochemistry, was observed in patients with ALK translocation compared to those with wt ALK and EGFR. Additionally, mRNA expression analysis of marker genes was conducted to estimate the abundance of 14 types of immune cell populations (including B-cells, CD45+, CD56dim, TCD8+, cytotoxic cells, CD8+ exhausted T-cells, macrophages, mast cells, neutrophils, NK cells, T-cells, Th1 and Treg cells). This analysis revealed a higher number of Treg cells in

Table 1. Studies on TIME composition in ALK translocated NSCLC.

Study	Study Objectives (parameters related to TIME)	ALK+ human samples (N)	Outcomes
Zeng C et al. [23]	PDL1, PD1, CD8, IFN-γ expression	33	• Low PDL1 expression • High PD1-positive $CD8^+$ T cells infiltration • Absent expression of IFN-γ RNA
D'Incecco A et al. [26]	PD1 PDL1 expression	10	• High PDL1 expression
Koh J et al. [27]	PD1 PDL1 expression	58	• PD-L1 expression in 81% • PDL1+ ALK+ associated with higher numbers of tumor-infiltrating PD1 + cells
Heo JY et al. [28]	PD-L1 expression, treatment outcomes, RNA expression level and cytolytic activity	14	• High PD-L1-positive rates • Low IFN-γ-related response
K Ota et al. [29]	PDL1 expression	NA*	• Upregulation of PDL1 by PI3K-AKT and MEK-ERK signaling pathways
Voena C et al. [30]	PD1, PDL1 expression TIME evaluation	NA°	• High numbers of PD1+ T cells • High expression of TIM-3 and LAG-3 • High infiltrating Treg cells
Gainor JF et al. [33]	PDL1 expression, CD8+ TILs evaluation	27	• Low rates of concurrent PD-L1 expression and CD8+ TILs
Budczies J et al. [34]	Immune-related gene expression profiling, TILs evaluation	31	• High Treg • Immunosuppressive TIME
Jiang B et al. [36]	Tumor genome mutation analysis. PDL1 expression and TILs evaluation.	84	• Co-occurring TP53/CDKN2A/B variations associated with high TMB • Immunosuppressive TIME
Zhang B et al. [31]	PDL1 expression, TILs evaluation	39	• High TCD4+ • Low rate of T cells expressing TIM-3-CD8+, CTLA4-CD8+, LAG3-CD8+, PD1-CD8+

Legend: IFN, interferon; TIME, tumor immune microenvironment; TIL, tumor infiltrating lymphocyte.
*ALK+ cell lines.
°ALK+ cell lines and mice.

patients with ALK translocations [34]. In this study, the investigation of cytokine and cytokine receptor expression differences between ALK-rearranged and ALK/EGFR wt patients was conducted using gene expression profiles. In ALK-translocated NSCLCs, higher expression levels of IL-2, TNFRSF4 (OX40), CXCL12, CCL2, and TNFSF13 (APRIL) were observed, while lower expression levels of IL-11, CXCL10, and CXCL11 were described [34].

An Asian population study assessed intratumoral immune cell composition through gene expression profiles in 11 Asian patients with ALK/EML4 fusion, finding that TIME were characterized by an increase in resting memory CD4+ T cells and a decrease in active memory cells [35]. Moreover, in a study examining the expression of CD8, interferon-γ (IFN-γ), and PD-1 via immunohistochemistry and RT-PCR in ALK-translocated lung adenocarcinoma samples from 25 patients undergoing resection, individuals with ALK rearrangements displayed a reduced density of CD8+ T cells in the tumor stroma. Notably, analysis of IFN-γ mRNA expression via RT-PCR in 10 patients, including 5 with ALK translocations and 5 with wt ALK, revealed IFN-γ expression only in wt ALK patients [23]. In this light, a reduced signature associated with interferon-γ response compared to ALK-negative NSCLC was evaluated through gene set enrichment analysis conducted on bulk RNA sequencing data from 14 patients with ALK-positive NSCLC [28].

The presence of co-alterations in ALK translocated lung cancer may also influence the immune microenvironment. In a retrospective analysis evaluating tumor samples from patients with ALK/RET/ROS1 rearranged NSCLC, TP53/CDKN2A/B co-alterations were associated with high levels of PD-L1 in the tumor area and reduced levels of CD8+, CD8+PD1-, and CD8+PD-L1- TILs, suggesting an immunosuppressive microenvironment [36].

3.1. Impact of ALK TKIs on TIME composition

Of interest, the modification of TIME composition can be induced by several ALK TKIs (Figure 2). Crizotinib and ceritinib have been shown to induce multiple markers of immunogenic cell death (ICD) in patient derived ALK+ NSCLC cell lines. This leads to heightened recognition of cancer cells by innate immune cells such as DCs and macrophages, promoting their differentiation which lead to recruit and stimulate a T cell response [37,38]. Interestingly, in the phase Ib clinical trial of 7 days of alectinib prior to atezolizumab in treatment-naive ALK + NSCLC patients, the CD8 T-cell count were increased post-alectinib run-in in seven out of nine paired biopsies collected at screening and day 7 of cycle 1; however, no clear association with response was found with CD8 detection [39].

Among studies evaluating microenvironment after resistance or response to ALK TKIs, we can deduce that resistance typically results in an immunosuppressive environment, while response to ALK TKIs lead to an immunoreactive microenvironment.

3.1.1. TIME and resistance to ALK TKI

Kim et al., analyzing PD-L1 expression in ALK-translocated NSCLC cell lines resistant to ALK TKIs crizotinib, ceritinib, and alectinib, reported that TKI resistance results in increased PD-

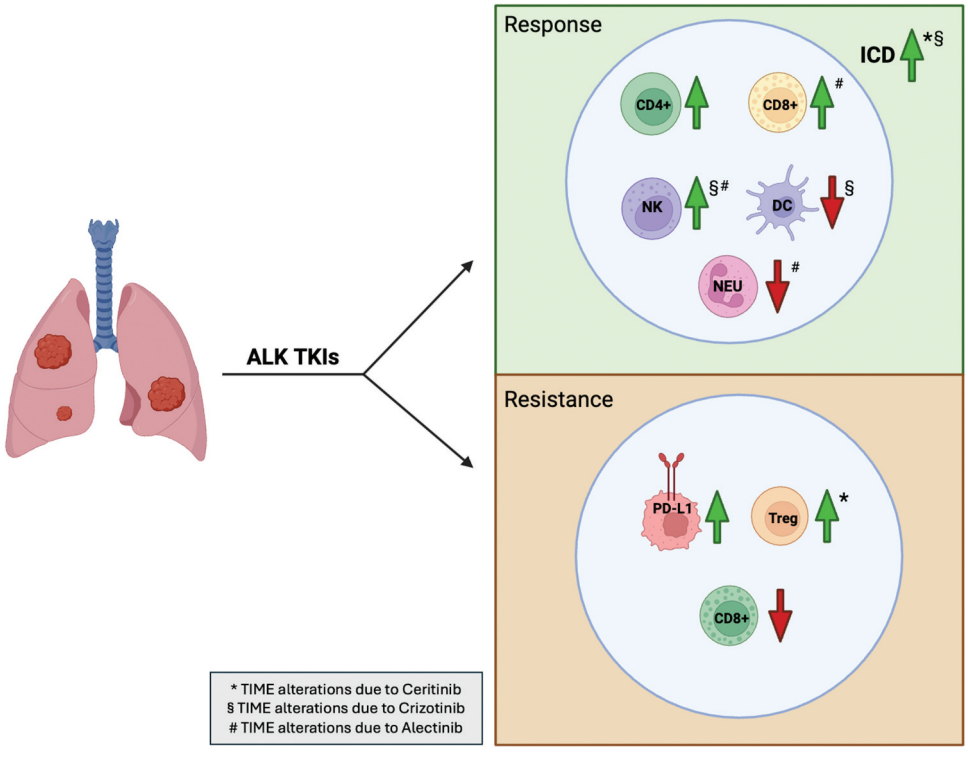

Figure 2. Modifications in tumor immunological microenvironment composition in ALK rearranged NSCLC induced by ALK TKIs.

In tissue samples from animal models or patients in response to ALK TKI treatments, an increase in TCD8+ CD3+ cells, CD4+ and natural killer cells was observed (upper panel). ALK translocated NSCLC samples that develop resistance to ALK TKI show a low presence of T CD8+, high presence of Treg in TIME and higher expression of PDL1 (lower panel).

Legend: TIME, tumor immune microenvironment; TKI, tyrosine kinase inhibitor; ICD, immunogenic cell death.

L1 expression. Transcriptomic analysis revealed differential expression of 20 genes compared to treatment-naïve ALK-translocated NSCLC cell lines, mostly involved in the immune system, suggesting the immune system's role in TKI resistance development [40].

In a preclinical study, phenotypic analyses on murine models of NSCLC harboring EML4-ALK translocation after resistance to ceritinib or ceritinib plus anti-PD1 revealed an increase in PD-L1-expressing tumor cells. However, this increase did not lead to rise in effector T-cells, as evidenced by the lack of significant changes in PD-1 or granzyme B expression in T-cells. Furthermore, ceritinib resistance was associated with an augmentation in Treg cells, identified through enhanced expression of Foxp3 and upregulation of genes related to Treg differentiation. In animal models exhibiting resistance to ceritinib, an elevation in the expression of the SOCS1 gene, crucial for maintaining Treg integrity and function, was observed [22]. An immunohistochemistry analysis on 11 treatment-naive patients and 16 patients crizotinib-resistance showed a statistically significant decrease in CD68 expression and a trend toward lower expression of CD3, CD8, and PD-1 in patients with TKI resistance [40].

Considering that PD-L1 expression and the presence of TILs have been correlated with clinical responses to PD-1/PD-L1 inhibitors, Gainor et al. used immunohistochemical and quantitative methods to determine the levels of tumor-infiltrating CD8+ lymphocytes in biopsies of 9 patients crizotinib resistant. Their analysis revealed no elevated expression of CD8+ TILs in any specimen and none of the patients showed simultaneous presence of CD8+ TILs and PD-L1 expression [33] (Figure 2). Recently, Angeles et al. investigated the potential of circulating cytokines as biomarkers in ALK positive NSCLC, through longitudinal serum samples from 38 patients undergoing TKIs therapy. While IL-6, IL-8, and IL-10 levels were significantly increased at disease progression, their baseline levels were not prognostic of the durable benefit of TKI therapy [41].

3.1.2. TIME and response to ALK TKI

Using orthotopic mouse models of ALK-translocated NSCLC treated with alectinib, Kleczko et al. demonstrated the crucial role of the adaptive immune response in achieving durable responses with ALK TKIs. While immunocompetent mice treated with alectinib showed deep and lasting responses, immunocompromised mice lacking functional *T*- and B-cells exhibited initial response followed by rapid progression within 3–4 weeks of alectinib. Comparative analysis between a mouse model achieving complete response and one achieving partial response revealed higher presence of tumor infiltrating CD3+CD8+ T cells and lower presence of neutrophils. Furthermore, after 4 days of treatment, there was an observed trend of CD8+ T cell increase and further decrease in neutrophils [42]. Single-cell RNA sequencing and multispectral tissue imaging were conducted on 49 clinical biopsies obtained from 30 patients with metastatic lung cancer, including 10 ALK-positive NSCLC. This analysis was performed prior and during targeted therapy, revealing increased T cell infiltration in TIME shortly after initiating TKI treatment [43]. Evaluation of TIME remodeling in tissue specimens from 8 ALK-translocated NSCLC responders to ALK TKI, using whole genome sequencing (WES) and RNA sequencing (RNA-seq), revealed increased infiltration of immune and cytotoxic cells only in not progressors patients. Furthermore, responders exhibited significant upregulation of genes promoting T-cell activation and differentiation. Utilizing bioinformatic techniques to identify immune cell subsets, an augmentation of cytotoxic cells within the tumor microenvironment, including CD8+ T-cells, gamma-delta T-cells, and NK cells, was reported [16].

Additionally, analysis of tissue samples from 14 patients before and after TKI treatment demonstrated that the total content of polymorphonuclear cells (in both tumor and stroma) and the ratio of polymorphonuclear cells to lymphocytes were negatively correlated with PFS, whereas such correlation was not observed when considering the sole lymphocyte count [42].

In a study involving surgical samples from 70 patients diagnosed with lung adenocarcinoma with ALK fusion, a downregulation of PD-L1 and HLA-I expression on the tumor cell membrane was reported, suggesting that ALK inhibition and its downstream signaling pathway appear to possibly reverse it [44].

Exposure to signaling molecules linked to M2 macrophages induces in vitro resistance to alectinib treatment via MET bypass signaling. Interestingly, treatment with crizotinib, an inhibitor targeting ALK and MET, reversed the resistance induced by exposure to M2 macrophage conditioned media [45].

In a neoadjuvant trial, several ALK TKIs (ensartinib, crizotinib, alectinib and ceritinib) demonstrated to remodel TIME by promoting the infiltration of cytotoxic CD8+ T cells and CD4+ T-helper cells, but not macrophages. Compared to resected specimens achieving pathological complete response (pCR) by neoadjuvant immunotherapy, higher levels of CD8+PD1+ T cells and lower levels of CD8+GranzymeB+ T cells were noticed in surgical specimen after ALK TKIs. Notably, one patient who experienced disease relapse showed highest level of CD8+PD1+ T cells in the pretreatment specimen, suggesting that baseline CD8+PD1+ T cells may play a critical role in influencing therapeutic outcomes [46].

Lastly, a study examining the impact of alectinib and crizotinib on human monocyte-derived dendritic cells (moDCs) through immunophenotyping via flow cytometry, migration, antigen uptake, and cytokine assays revealed notable differences. Crizotinib-treated DCs exhibited reduced activation markers, such as CD83, diminished chemokine-guided migration, decreased antigen uptake, and lower production of pro-inflammatory cytokines, notably Interleukin-12. In contrast, the immunosuppressive effects of alectinib were considerably less pronounced [47] (Figure 2).

4. Immunotherapy in patients with ALK-positive NSCLC

Based on previously reported data, TIME in ALK-mutated NSCLC is characterized by a slight activation of the immune system against the tumor ('immunologically cold') [48]. A major goal in current anticancer drug development is to increase the infiltration and activation of T cells within the TIME, due to their role in tumor control and elimination [49].

One approach to achieve this is to induce ICD within tumor cells, thereby stimulating the adaptive immune system. ICD contributes to increased identification of tumor cells by innate immune cells, such as DCs and macrophages, and promotes their differentiation into activated phenotypes able to recruit and stimulate a T cell response [37]. In this light, theoretically, a therapeutic approach aimed at transforming the TIME in a more immunologically active environment could allow for better treatment efficacy.

To date, findings from a subgroup analysis of a prospective single-arm study and two retrospective studies suggest an objective response rate of 0% in NSCLC patients with ALK rearrangement treated with anti-PD-(L)1 antibodies as monotherapy [33,50–52]. Conversely, anedoctical case reports reported positive response to immunotherapy treatment after exhausting therapeutic options with TKIs. A patient with an ALK G1202R mutation, after two different TKIs and platinum-based chemotherapy, received pembrolizumab, achieving a partial response for 9 cycles [53]. Another patient underwent treatment with ceritinib, followed by a regimen combining platinum and bevacizumab before experiencing disease progression. Following this, the patient received nivolumab as third-line therapy, resulting in a complete response observed in radiographic imaging for around 16 months [54].

A recent retrospective study, among 216 patients affected by NSCLC with oncogenic driver alterations other than EGFR NSCLC, included 14 patients with ALK positive NSCLC treated with immunotherapy both as monotherapy and in combination with chemotherapy, in both first and second line. Overall, immunotherapy efficacy resulted modest, confirming that ALK TKIs should remain the first-choice treatment option [55].

Despite the combination of TKIs and immunotherapy seemed to be an approach capable of increasing responsiveness to TKIs and thus a promising way forward for patients with ALK-positive NSCLC, the available data derived from early phase clinical trials did not demonstrate activity of this combination.

Crizotinib plus nivolumab was initially tested in the CheckMate 370 but, due to early serious adverse events, the trial stopped [56]. Moreover, the combination of ceritinib and nivolumab in first and subsequent lines also produced a response rate of 69% and 35%, respectively, similar to the results obtained by ceritinib monotherapy [57–59].

Similarly, a trial evaluating the combination of Ipilimumab, an anti-CTLA-4 antibody, and EGFR and ALK TKIs, was prematurely discontinued due to toxicities observed in both patient cohorts (in ALK+ patients, grade 3 hypophysitis and grade 2 pneumonia) without response data reported [60]. Moreover, alectinib and atezolizumab combination demonstrated promising activity with increased toxicity compared to the use of each agent individually [39].

Based on the available findings, novel generation ALK TKIs seems to have lower toxicity when combined with immunotherapy but with no clear evidence of increased efficacy. In this light, in the JAVELIN Lung 101 study, the combination of avelumab and lorlatinib in 28 previously treated patients reported no dose-limiting toxic events (DLTs) or grade 4 or 5 adverse events with a response rate of 46.4% [61,62]. Of note, definitive conclusions about the treatment's activity could not be established and, consequently, more extensive studies are necessary to further validate these findings.

Finally, to date, immunotherapy combined with chemotherapy represents the standard of care for non-oncogene addicted metastatic NSCLC [63]. In the Impower130 trial evaluating atezolizumab plus chemotherapy as first-line treatment, patients enrolled in the experimental arm presenting EGFR or ALK alterations (7%, $n = 32$) demonstrated no survival benefit comparing to patients treated with chemotherapy alone [64]. A subgroup analysis of IMpower150 trial, in ALK positive patients after progression or intolerance to at least 1 TKI, demonstrated a slight improvement in PFS and overall survival (OS) of atezolizumab and bevacizumab plus carboplatin plus paclitaxel [65].

Overall, the PD-L1–PD-1 axis inhibition provides only limited benefit in patients with NSCLC displaying ALK alterations (Table 2).

5. Future perspectives

Based on the previous evidence, immunotherapy, alone or in combination with other treatments, have not yet found a place in the treatment landscape of patients with ALK-rearranged NSCLC.

In this light, based on the subgroup results obtained by IMpower150, combination studies with immunotherapy (anti PD-1 or PD-L1), chemotherapy (and antiangiogenic drugs) have been conducted or are currently recruiting [66].

Atezolizumab and platinum doublet with or without bevacizumab have been investigated in oncogene-addicted (EGFR or ALK positive) patients in some phase II and III studies. Bylicki et al., analyzing data from 13 ALK-translocated patients, demonstrated that atezolizumab with or without bevacizumab and platinum-pemetrexed treatment achieved promising activity after tyrosine kinase inhibitor failure, with an acceptable safety profile [67].

Additionally, a phase 3 study is currently ongoing to evaluate the efficacy of atezolizumab in combination with carboplatin, paclitaxel, and bevacizumab compared to treatment with pemetrexed and cisplatin in TKI pretreated patients with activating EGFR mutation or ALK translocation (NCT03991403).

Interestingly, a case series involving two patients with ALK-rearranged NSCLC, who had previously undergone multiple ALK TKI (including lorlatinib) and/or chemotherapy, showed promising outcomes when treated with a combination of bevacizumab and lorlatinib. This combination was well tolerated and could offer benefits for lorlatinib-resistant patients [68].

Pembrolizumab with bevacizumab and chemotherapy in patients with ALK-positive NSCLC after progression on alectinib is currently under evaluation in a small phase II trial (NCT05266846). The NCT04425135 study is evaluating the combination of camrelizumab, a PD-1 inhibitor, together with apatinib, a small molecule inhibitor of vascular endothelial growth factor receptor 2, and carboplatin-pemetrexed (Table 3).

Table 2. Immunotherapy trials in patients with ALK-positive NSCLC.

	Study	Drug	Study design	Stage disease	N ALK+ patients	N TKI pretreated/ N TKI naive	Safety (% AEs ≥ G3)	ORR (%)	mPFS (months)
ICIs monotherapy	Gainor et al., 2016 [33]	Any anti-PD-(L)1 antibody	Retrospective study	Advanced	6	6/0	NR	0	NR
	Garassino et al., 2018 [50]	Durvalumab	Phase II, open label	IIIB/IV	15	15/0	NR	0	NR
	Mazieres et al., 2019 [51]	Any anti-PD-(L)1 antibody	Retrospective study	Advanced	19	19/0	NR	0	2
ICIs plus TKIs	Shaw et al., 2018 [61]	Lorlatinib + Avelumab	Phase Ib, open label	Advanced	28	27/0	53.6%	46%	NR
	Spigel et al., 2019 [56]	Crizotinib + Nivolumab	Phase I/II, open label	Advanced	13	13/0	61.5%	38	NR
	Chalmers et al., 2019 [60]	Crizotinib + Ipilimumab	Phase I, open label	II-IV	3	3/0	33.3%	NE	NE
	Felip et al., 2020 [57]	Ceritinib + Nivolumab	Phase Ib, open label	IIIB/IV	38	20/16	86%	44%	4.6[#]
	Kim et al., 2022 [39]	Alectinib + Atezolizumab	Phase Ib, open label	Advanced	21	2/19	57%	86%	NE
ICIs plus CT	West et al., 2019 [64]	Atezolizumab + CBDCA + Nab-Paclitaxel	Phase III, randomized	Advanced	32*	32*/0	NR	NR	7*
ICIs plus CT and antiangiogenic drug	Socinski et al., 2021 [66]	Atezolizumab + CBDCA + Paclitaxel + Bevacizumab	Phase III, randomized	Advanced	13	13/0	58.5%[α]	NR	8.3

Legend: *ALK+EGFR positive patients; [#]In TKI pretreated patients; [α]In all patients. ICIs, immune checkpoint inhibitors; TKIs, tyrosine kinase inhibitors, CT, chemotherapy; CBDCA Carboplatin; NR, not reported; NE, not estimable.

5.1. Potential treatment strategies targeting TIME in ALK positive NSCLC

Mounting attention has shifted toward targeting players in the TIME. Below, we will analyze some of the novel therapeutic approaches (Table 3).

The initial findings regarding a potential DNA-based vaccine targeting ALK come from a study that analyzed the growth of ALK-positive lung tumors in murine models. The vaccine induced a strong and specific immune response, demonstrating activity even in combination with TKIs directly targeting ALK [30]. Of note, in a recent preclinical trial, the authors demonstrated that the poor immunogenicity of ALK positive disease could be improved by enhancing the priming of ALK-specific CD8+ T cells through vaccination with a single ALK peptide. This vaccination seems to 1) increase the numbers of intratumoral ALK-specific CD8+ T cells, 2) delay tumor progression, 3) extend overall survival and 4) cure a subset of mice when combined with lorlatinib, preventing also central nervous system progression [69].

Additionally, due to the evidence that the tumor microenvironment in ALK-rearranged mice was mostly immunosuppressive (great amount of Tregs) [70], benefits of combined

Table 3. Future perspectives and clinical trials in NSCLC ALK-positive.

	ClinicalTrials.gov Identifier	Study design	Therapeutic Agents	Primary outcome	Status	Estimated study completation date
ICIs plus CT ± antiangiogenic drug	NCT04042558	Phase II	Atezolizumab + platinum (carboplatin/cisplatin), pemetrexed ± bevacizumab	Objective response rate	Recruiting	June 2024
	NCT03991403	Phase III	Atezolizumab + carboplatin, paclitaxel + bevacizumab VS pemetrexed + cisplatin/carboplatin	Progression-free survival	Active, not recruiting	March 2024
	NCT05266846	Phase II	Pembrolizumab + Bevacizumab + Pemetrexed + Carboplatin	Progression-free survival	Not yet recruiting	February 2025
	NCT04425135	Phase II	Camrelizumab + pemetrexed + apatinib mesylate	Objective response rate	Not yet recruiting	January 2025
Vaccine	NCT05950139	Phase I/II	Peptide vaccine	Safety	Not yet recruiting	July 2029
	NCT05195619	Phase Ib,	Personalized DC vaccine in combination with low-dose cyclophosphamide	Patients with one dose of vaccine Safety Treatment-limiting toxicities	Recruiting	September 2024
TILs	NCT03645928	Phase II	TIL + pembrolizumab VS TIL as a single agent therapy	Objective Response Rate Safety	Recruiting	December 2024
	NCT04872634	Phase I/IIa	SNK01 (NK Cells) + CT ± Cetuximab	Maximum Tolerated Dose Safety	Unknown	NA
	NCT05681780	Phase I/II	CD40L TILs + Nivolumab	Safety	Recruiting	July 2025

Legend:ICIs, Immune Checkpoint Inhibitors; CT, chemotherapy; DC, dendritic cells; VS, versus; TIL, tumor infiltrating lymphocytes; NK, Natural killer; NA, not available.

therapy with ALK TKIs and ALK vaccine may be enhanced by immunotherapies, such as anti-PD-1/PD-L1 and anti-CTLA4, through block immune checkpoints [71,72], or anti-CD25 antibodies through Treg depletion [73].

One interesting approach is based on the use of DCs vaccination. DCs can effectively present tumor antigens to initiate T-cell-mediated immune responses, and the interaction of DCs with other immune cells within the TIME strengthens anti-tumor immune signaling. The direct interaction of DCs with tumor cells through antigen uptake and presentation, coupled with their ability to interface with immune cells in the tumor environment to promote anti-tumor immune signaling, makes them an ideal candidate for cellular vaccination.

Interestingly, to date, two clinical trials are evaluating vaccination in patients with ALK-positive NSCLC. The first one focuses on the use of personalized DCs vaccines (NCT05195619), while the other is a phase II trial that combined DCs vaccine with chemotherapy (NCT05950139).

Adoptive cell therapy with TILs represents an autologous adoptive immunotherapy strategy involving in vitro expansion of T lymphocytes and in vivo reinfusion in association with interleukin 2 (IL-2) after chemotherapy [74].

Adoptive cell therapy using TILs constitutes a novel approach in immunotherapy for solid malignancies, including lung cancer. In a phase I clinical trial, TILs were administered alongside nivolumab to patients with metastatic NSCLC. Of note, three patients treated with TILs and nivolumab experienced a radiological response, including one case of complete response in a patient with EGFR+ NSCLC osimertinib-resistant [75].

A large multicenter phase II trial, evaluating the efficacy of TIL therapy with and without the immunotherapy, is currently ongoing, also in patients affected by ALK positive NSCLC (NCT03645928). Other forms of adoptive cell therapies currently under investigation include autologous NK cell therapy, administered alone or in combination with chemotherapy. This phase I/IIa study foresees the enrollment exclusively of patients with lung cancer who have progressed after treatment with TKIs, including those with ALK positive NSCLC (NCT04872634).

The CD47, usually overexpressed in lung cancer, is a widely expressed cell surface molecule that prevents phagocytosis of target cells by innate immune system through its interaction with signal regulatory protein alpha (SIRPα) [76].

The recognition and phagocytosis, by macrophages, is partly influenced by the expression of CD47 on the tumor cells. Data from early-phase clinical trials suggest that monotherapy has limited efficacy, while a greater response is observed when used in combination [77]. Interestingly, the adverse event profile of therapies targeting the CD47/SIRPα axis does not include immune-related adverse events classically associated with PD-1/PD-L1/CTLA-4 checkpoint inhibition [77,78].

In vitro, alectinib-resistant H2228 cells lines generated two distinct cell populations based on CD47 expression [79]. The inoculation of the CD47HiH2228 or CD47LoH2228 subtypes in nude mice revealed the significant tumorigenicity of the CD47Hi subpopulation [76]. Furthermore, mice bearing H3122 (a human ALK-positive NSCLC cell line) tumors were treated with lorlatinib and anti-CD47 combination, demonstrated tumor reduction and duration of response [80].

An important role as a promoter of the T cell response could be played by modified versions of interleukin-2, binding to effector T cells and not interacting with Treg [81].

In this light, modified IL-2 could favor the acquisition of effector functions of T cells already present in the TIME of ALK + NSCLC, while avoiding the proliferation and function of Treg cells [16,33,43]. A phase I study of modified IL-2 plus nivolumab, including 5 patients with treatment-naive metastatic NSCLC, showed an increase in CD8+ T cells in the TIME during treatment and no increase in Treg cells [82].

Oncolytic virus therapy is based on the use of viral agents that can selectively replicate inside tumor cells, inducing ICD. In order to improve anti-tumor efficacy, viral agents can be easily modified in vitro to express cytokines/chemokines [83].

A strategy, already available in melanoma patients, is represented by the talimogene laherparepvec (T-VEC), a modified herpes virus that stimulates the macrophage granulocyte colony-stimulating factor to recruit and activate antigen-presenting cells [84]. An increase in tumor antigen-specific T-cells, accompanied by a reduction in Treg cells and monocyte-derived suppressor cells, was demonstrated in lesions treated with T-VEC [85].

Addressing the heterogeneity of the tumor immune environment (TIME) through the induction of immunogenic cell death and subsequent T cell-dependent anti-tumor responses represents one of the main objectives of T cells therapy equipped with chimeric antigen receptor (CAR-T) [86,87].

The infiltration of CAR-T cells into solid tumor tissues remains a prerequisite for their antitumor function, relying on their efficient and specific trafficking capabilities. Mismatched chemokine-chemokine receptor pairs, down-regulation of adhesion molecules, aberrant vascularization, immunosuppressive TIME, and the anatomical location of immune effector cells can all contribute to the poor homing of these cells [88]. Limited trafficking within a solid tumor compared to hematologic diseases and restricted T-cell activity in the microtumoral environment pose significant challenges [89,90]. To overcome issues associated with CAR-T cell entry into the solid tumor environment or penetration into the tumor ECM, Caruana et al. modified CAR-T cells to express heparanase (HPSE), an enzyme that aids in degrading components of the tumor ECM, thus promoting T-cell invasion and antitumor activity [91].

Several trials, exploring different potential targets for CAR-T therapy in NSCLC, such as EGFR, HER2, mesothelin, CD80/CD86, carcinoembryonic antigen (CEA).

For example, a phase I clinical trial is being conducted to investigate the safety, tolerability, and pharmacokinetic properties of the CAR-T C-13-60 cells. The study aims to evaluate the efficacy of the drug in late-stage malignant solid tumors positive for CEA (NCT06043466).

In addition, another clinical study is assessing the safety and tolerability of autologous mesothelin-targeted chimeric antigen receptor (MSLN-CAR) T cells engineered to secrete PD-1 nanobodies in patients with solid tumors (including ALK-translocated NSCLC) (NCT04489862).

6. Conclusions

Recognizing the pivotal role of TIME in driving cancer progression marks a significant paradigm shift, moving beyond a singular focus on tumor cells to embrace the complexity of the entire tumor ecosystem. As we endeavor to identify effective immune-modulating therapies for ALK positive NSCLC in the future, it becomes imperative to delve deeper into several key areas. There is a pressing need to gain a comprehensive understanding of how TKIs therapy influences the intricate dynamics of the TIME specifically within ALK positive NSCLC. Additionally, elucidating the mechanisms by which residual disease manages to evade immune detection represents another critical avenue of investigation. Furthermore, determining the specific infiltrating immune cell populations that play pivotal roles in enhancing TKI response and ultimately improving patient outcomes is paramount. By addressing these fundamental questions, we can pave the way for the development of more targeted and efficacious therapeutic strategies tailored to the unique immunological landscape of ALK positive NSCLC.

7. Expert opinion

As previously observed, the TIME comprises an intricate system of interconnected components. Its characteristics may depend on various factors, particularly the genetic landscape of the tumor, which can also influence the immune response. Furthermore, the presence of oncogenic driver mutations within tumor cells can significantly impact the composition and features of TIME [92]. For instance, EGFR mutant NSCLC often display an immunosuppressive TIME characterized by reduced infiltration of immune cells [21]. Additionally, co-occurring mutations play a role in shaping the immune tumor microenvironment. In NSCLC cases with KRAS mutations and concurrent mutations within the P53 gene, distinct TIME characteristics and clinical responses to immunotherapy have been observed [93]. Conversely, the co-occurrence of mutations in STK11 or KEAP1 results in an immunosuppressive microenvironment and is generally associated with an unfavorable response to ICIs [93,94]. On the other hand, alterations in TP53/CDKN2A/B and co-occurring ALK/RET/ROS1 rearrangements are associated with an immunosuppressive microenvironment and a worse prognosis [36].

In addition to the genetic profile, the treatments can exert a notable influence on the TIME, which in turn can impact treatment response [95]. Particularly, the evidence of the correlation between response duration to anti-ALK TKIs (such as alectinib) and the presence of an active immune TIME appears to confirm its role in the durability of TKI responses [42]. These findings are corroborated by additional evidence indicating that increased infiltration of immune and cytotoxic cells has been identified in patients who have exhibited a positive response to anti-ALK TKIs [16]. Conversely, an escalation in immune suppression has been observed in patients who experience disease progression to anti-ALK TKIs [40].

Hence, forthcoming investigations may direct attention toward elucidating the intricate interplay between TKIs and TIME. A broad comprehension of how the TIME evolves under the selective pressure of anti-ALK TKIs could hold pivotal significance in refining therapeutic strategies and treatment sequencing for patients.

Specifically, the role of rebiopsy at the time of disease progression assumes newfound importance. Beyond assessing molecular mechanisms of resistance to anti-ALK TKIs, it could serve as a crucial tool for investigating the dynamic landscape of TIME. The insights collected from such analyses could prove invaluable, not only for advancing scientific understanding and research endeavors but also potentially for guiding the optimization of subsequent therapeutic interventions.

In light of this consideration, given the pivotal role of immunity within this treatment context, the theoretical synergy between immunotherapy and anti-ALK targeted therapy could offer new treatment possibilities. However, while the combination of immunotherapy and TKIs has shown convincing efficacy in certain scenarios [53,54], it has concurrently revealed significant toxicity concerns in numerous cases [57–59]. This toxicity profile has, in fact, hindered the widespread adoption of this therapeutic combination thus far.

The utilization of immunotherapy continues to be a primary area of investigation in these patients. It is increasingly recognized that combining novel agents aimed at modulating the TIME may hold promise for achieving effective treatment outcomes while maintaining acceptable levels of toxicity. Consequently, there is a burgeoning interest in exploring these combination approaches in clinical research. Presently, numerous studies are actively underway, investigating the potential efficacy and safety of such regimens in treating various solid tumors, including ALK-positive NSCLC.

Modified IL-2, CAR-T cell therapies, and the combination of novel immune agents with anti-ALK TKIs are just examples within the important array of treatments that are currently under investigation [80,82,91].

These studies aim to elucidate the mechanisms underlying the synergistic effects of these agents and their potential to improve patient outcomes in the clinical setting. Such endeavors represent crucial steps toward advancing the treatment landscape for patients with ALK translocated NSCLC.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Gregg JP, Li T, Yoneda KY. Molecular testing strategies in non-small cell lung cancer: optimizing the diagnostic journey. Transl Lung Cancer Res. 2019;8(3):286–301. doi: 10.21037/tlcr.2019.04.14
2. Yatabe Y. Molecular pathology of non-small cell carcinoma. Histopathology. 2024;84(1):50–66. doi: 10.1111/his.15080
3. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007;448(7153):561–566. doi: 10.1038/nature05945
4. Iragavarapu C, Mustafa M, Akinleye A, et al. Novel ALK inhibitors in clinical use and development. J Hematol Oncol. 2015;8(1):17. doi: 10.1186/s13045-015-0122-8
5. Rothenstein JM, Chooback N. ALK inhibitors, resistance development, clinical trials. Curr Oncol. 2018;25(Suppl 1):S59–s67. doi: 10.3747/co.25.3760
6. Caccese M, Ferrara R, Pilotto S, et al. Current and developing therapies for the treatment of non-small cell lung cancer with ALK abnormalities: update and perspectives for clinical practice. Expert Opin Pharmacother. 2016;17(17):2253–2266. doi: 10.1080/14656566.2016.1242578
7. Camidge DR, Kim HR, Ahn MJ, et al. Brigatinib versus Crizotinib in ALK Inhibitor-naive advanced ALK-Positive NSCLC: final results of phase 3 ALTA-1L Trial. J Thorac Oncol. 2021;16(12):2091–2108. doi: 10.1016/j.jtho.2021.07.035
8. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus crizotinib in untreated ALK-Positive non-small-cell lung cancer. N Engl J Med. 2017;377(9):829–838. doi: 10.1056/NEJMoa1704795
9. Shaw AT, Bauer TM, de Marinis F, et al. First-line lorlatinib or crizotinib in advanced ALK-Positive lung cancer. N Engl J Med. 2020;383(21):2018–2029. doi: 10.1056/NEJMoa2027187
10. Soria JC, Tan DSW, Chiari R, et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. Lancet. 2017;389(10072):917–929. doi: 10.1016/S0140-6736(17)30123-X
11. Pan Y, Deng C, Qiu Z, et al. The resistance mechanisms and treatment strategies for ALK-rearranged non-small cell lung cancer. Front Oncol. 2021;11:713530. doi: 10.3389/fonc.2021.713530
12. Xue Y, Hou S, Ji H, et al. Evolution from genetics to phenotype: reinterpretation of NSCLC plasticity, heterogeneity, and drug resistance. Protein Cell. 2017;8(3):178–190. doi: 10.1007/s13238-016-0330-1
13. Yuan S, Norgard RJ, Stanger BZ. Cellular plasticity in cancer. Cancer Discov. 2019;9(7):837–851. doi: 10.1158/2159-8290.CD-19-0015
14. Bianco A, Perrotta F, Barra G, et al. Prognostic factors and biomarkers of responses to immune checkpoint inhibitors in lung cancer. Int J Mol Sci. 2019;20(19):4931. doi: 10.3390/ijms20194931
15. Selenz C, Compes A, Nill M, et al. EGFR inhibition strongly modulates the tumour immune microenvironment in EGFR-Driven non-small-cell lung cancer. Cancers (Basel). 2022;14(16):3943. doi: 10.3390/cancers14163943
16. Fang Y, Wang Y, Zeng D, et al. Comprehensive analyses reveal TKI-induced remodeling of the tumor immune microenvironment in EGFR/ALK-positive non-small-cell lung cancer. Oncoimmunology. 2021;10(1):1951019. doi: 10.1080/2162402X.2021.1951019
17. Lim JU, Lee E, Lee SY, et al. Current literature review on the tumor immune micro-environment, its heterogeneity and future perspectives in treatment of advanced non-small cell lung cancer. Transl Lung Cancer Res. 2023;12(4):857–876. doi: 10.21037/tlcr-22-633
18. Chen S, Tang J, Liu F, et al. Changes of tumor microenvironment in non-small cell lung cancer after TKI treatments. Front Immunol. 2023;14:1094764. doi: 10.3389/fimmu.2023.1094764
19. Jin Z, Zhou Q, Cheng JN, et al. Heterogeneity of the tumor immune microenvironment and clinical interventions. Front Med. 2023;17(4):617–648. doi: 10.1007/s11684-023-1015-9
20. Belluomini L, Dodi A, Caldart A, et al. A narrative review on tumor microenvironment in oligometastatic and oligoprogressive non-small cell lung cancer: a lot remains to be done. Transl Lung Cancer Res. 2021;10(7):3369–3384. doi: 10.21037/tlcr-20-1134
21. Otano I, Ucero AC, Zugazagoitia J, et al. At the crossroads of immunotherapy for oncogene-addicted subsets of NSCLC. Nat Rev Clin Oncol. 2023;20(3):143–159. doi: 10.1038/s41571-022-00718-x
22. Pyo KH, Lim SM, Park CW, et al. Comprehensive analyses of immunodynamics and immunoreactivity in response to treatment in ALK-positive non-small-cell lung cancer. J Immunother Cancer. 2020;8(2):e000970. doi: 10.1136/jitc-2020-000970
23. Zeng C, Gao Y, Xiong J, et al. Tumor-infiltrating CD8(+) T cells in ALK-positive lung cancer are functionally impaired despite the absence of PD-L1 on tumor cells. Lung Cancer. 2020;150:139–144. doi: 10.1016/j.lungcan.2020.10.009
• **ALK traslocated tumor microenvironment suppresses the immune function of CD8+ T cells through a PD-1/PD-L1-independent mechanism.**
24. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol. 2020;20(11):651–668. doi: 10.1038/s41577-020-0306-5
25. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015;372(21):2018–2028. doi: 10.1056/NEJMoa1501824
26. D'Incecco A, Andreozzi M, Ludovini V, et al. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. Br J Cancer. 2015;112(1):95–102. doi: 10.1038/bjc.2014.555
27. Koh J, Jang JY, Keam B, et al. EML4-ALK enhances programmed cell death-ligand 1 expression in pulmonary adenocarcinoma via hypoxia-inducible factor (HIF)-1α and STAT3. Oncoimmunology. 2016;5(3):e1108514. doi: 10.1080/2162402X.2015.1108514
28. Heo JY, Park C, Keam B, et al. The efficacy of immune checkpoint inhibitors in anaplastic lymphoma kinase-positive non-small cell lung cancer. Thorac Cancer. 2019;10(11):2117–2123. doi: 10.1111/1759-7714.13195
29. Ota K, Azuma K, Kawahara A, et al. Induction of PD-L1 expression by the EML4-ALK oncoprotein and downstream signaling pathways in non-small cell lung cancer. Clin Cancer Res. 2015;21(17):4014–4021. doi: 10.1158/1078-0432.CCR-15-0016
•• **ALK fusion upregulate PD-L1 by activating PI3K-AKT and MEK-ERK signaling pathways in NSCLC.**
30. Voena C, Menotti M, Mastini C, et al. Efficacy of a cancer vaccine against ALK-Rearranged lung tumors. Cancer Immunol Res. 2015;3(12):1333–1343. doi: 10.1158/2326-6066.CIR-15-0089
31. Zhang B, Zeng J, Zhang H, et al. Characteristics of the immune microenvironment and their clinical significance in non-small cell lung cancer patients with ALK-rearranged mutation. Front Immunol. 2022;13:974581. doi: 10.3389/fimmu.2022.974581
32. Pan Y, Liu X, Zhang W, et al. Association of PD-L1 expression with efficacy of alectinib in advanced NSCLC patients with ALK fusion. Lung Cancer. 2023;181:107233. doi: 10.1016/j.lungcan.2023.107233
33. Gainor JF, Shaw AT, Sequist LV, et al. EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: a retrospective analysis. Clin Cancer Res. 2016;22(18):4585–4593. doi: 10.1158/1078-0432.CCR-15-3101
34. Jiang J, Kirchner M, Kluck K, et al. Deciphering the immunosuppressive tumor microenvironment in ALK- and EGFR-positive lung

adenocarcinoma. Cancer Immunol Immun. 2022;71(2):251–265. doi: 10.1007/s00262-021-02981-w
35. Jin R, Liu C, Zheng S, et al. Molecular heterogeneity of anti-PD-1/PD-L1 immunotherapy efficacy is correlated with tumor immune microenvironment in East Asian patients with non-small cell lung cancer. Cancer Biol Med. 2020;17(3):768–781. doi: 10.20892/j.issn.2095-3941.2020.0121
36. Jiang B, Hu L, Dong D, et al. TP53 or CDKN2A/B covariation in ALK/RET/ROS1-rearranged NSCLC is associated with a high TMB, tumor immunosuppressive microenvironment and poor prognosis. J Cancer Res Clin Oncol. 2023;149(12):10041–10052. doi: 10.1007/s00432-023-04924-7
37. Fucikova J, Kepp O, Kasikova L, et al. Detection of immunogenic cell death and its relevance for cancer therapy. Cell Death Dis. 2020;11(11):1013. doi: 10.1038/s41419-020-03221-2
38. Liu P, Zhao L, Pol J, et al. Crizotinib-induced immunogenic cell death in non-small cell lung cancer. Nat Commun. 2019;10(1):1486. doi: 10.1038/s41467-019-09415-3
39. Kim DW, Gadgeel S, Gettinger SN, et al. Brief report: safety and antitumor activity of alectinib plus atezolizumab from a phase 1b study in advanced ALK-Positive NSCLC. JTO Clin Res Rep. 2022;3(8):100367. doi: 10.1016/j.jtocrr.2022.100367
40. Kim SJ, Kim S, Kim DW, et al. Alterations in PD-L1 expression associated with acquisition of resistance to ALK inhibitors in ALK-Rearranged lung cancer. Cancer Res Treat. 2019;51(3):1231–1240. doi: 10.4143/crt.2018.486
41. Angeles AK, Janke F, Daum AK, et al. Integrated circulating tumour DNA and cytokine analysis for therapy monitoring of ALK-rearranged lung adenocarcinoma. Br J Cancer. 2023;129(1):112–121. doi: 10.1038/s41416-023-02284-0
42. Kleczko EK, Hinz TK, Nguyen TT, et al. Durable responses to alectinib in murine models of EML4-ALK lung cancer requires adaptive immunity. NPJ Precis Oncol. 2023;7(1):15. doi: 10.1038/s41698-023-00355-2
43. Maynard A, McCoach CE, Rotow JK, et al. Therapy-induced evolution of human lung cancer revealed by single-cell RNA sequencing. Cell. 2020;182(5):1232–51.e22. doi: 10.1016/j.cell.2020.07.017
44. Mu D, Guo J, Yu W, et al. Downregulation of PD-L1 and HLA-I in non-small cell lung cancer with ALK fusion. Thorac Cancer. 2022;13(8):1153–1163. doi: 10.1111/1759-7714.14372
45. Mandell M, Priest K, Le A, et al. Abstract 655: macrophage mediated resistance to TKI therapy in ALK fusion positive non-small cell lung cancer. Cancer Res. 2022;82(12_Supplement):655–. doi: 10.1158/1538-7445.AM2022-655
46. Zheng N, Zhang Y, Zeng Y, et al. Pathological response and tumor immune microenvironment remodeling upon neoadjuvant ALK-TKI treatment in ALK-Rearranged non-small cell lung cancer. Target Oncol. 2023;18(4):625–636. doi: 10.1007/s11523-023-00981-7
47. Heine A, Held SAE, Daecke SN, et al. Spoilt for choice: different immunosuppressive potential of anaplastic lymphoma kinase inhibitors for non small cell lung cancer. Front Immunol. 2023;14:1257017. doi: 10.3389/fimmu.2023.1257017
48. Schenk EL. Narrative review: immunotherapy in anaplastic lymphoma kinase (ALK)+ lung cancer-current status and future directions. Transl Lung Cancer Res. 2023;12(2):322–336. doi: 10.21037/tlcr-22-883
49. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov. 2019;18(3):197–218. doi: 10.1038/s41573-018-0007-y
50. Garassino MC, Cho BC, Kim JH, et al. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (ATLANTIC): an open-label, single-arm, phase 2 study. Lancet Oncol. 2018;19(4):521–536. doi: 10.1016/S1470-2045(18)30144-X
51. Mazieres J, Drilon A, Lusque A, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: results from the IMMUNOTARGET registry. Ann Oncol. 2019;30(8):1321–1328. doi: 10.1093/annonc/mdz167
52. Riudavets M, Auclin E, Mosteiro M, et al. Durvalumab consolidation in patients with unresectable stage III non-small cell lung cancer with driver genomic alterations. Eur J Cancer. 2022;167:142–148. doi: 10.1016/j.ejca.2022.02.014
- **Limited activity of durvalumab in patients with stage III unresectable EGFR/BRAF mutant and ALK positive NSCLC.**
53. Shimada M, Tamura A, Yokosuka K, et al. A successful pembrolizumab treatment case of lung adenocarcinoma after becoming resistant to ALK-TKI treatment due to G1202R mutation. Respir Investig. 2018;56(4):365–368. doi: 10.1016/j.resinv.2018.04.004
54. Baldacci S, Grégoire V, Patrucco E, et al. Complete and prolonged response to anti-PD1 therapy in an ALK rearranged lung adenocarcinoma. Lung Cancer. 2020;146:366–369. doi: 10.1016/j.lungcan.2020.05.008
55. Tian T, Li Y, Li J, et al. Immunotherapy for patients with advanced non-small cell lung cancer harboring oncogenic driver alterations other than EGFR: a multicenter real-world analysis. Transl Lung Cancer Res. 2024;13(4):861–874. doi: 10.21037/tlcr-24-116
56. Spigel DR, Reynolds C, Waterhouse D, et al. Phase 1/2 study of the safety and tolerability of nivolumab plus crizotinib for the first-line treatment of anaplastic lymphoma kinase translocation - positive advanced non-small cell lung cancer (CheckMate 370). J Thorac Oncol. 2018;13(5):682–688. doi: 10.1016/j.jtho.2018.02.022
57. Felip E, de Braud FG, Maur M, et al. Ceritinib plus nivolumab in patients with advanced ALK-rearranged non-small cell lung cancer: results of an open-label, multicenter, phase 1B study. J Thorac Oncol. 2020;15(3):392–403. doi: 10.1016/j.jtho.2019.10.006
58. Cho BC, Obermannova R, Bearz A, et al. Efficacy and Safety of Ceritinib (450 mg/d or 600 mg/d) with food versus 750-mg/d fasted in patients with ALK Receptor Tyrosine Kinase (ALK)-Positive NSCLC: primary efficacy results from the ASCEND-8 study. J Thorac Oncol. 2019;14(7):1255–1265. doi: 10.1016/j.jtho.2019.03.002
59. Chow LQM, Barlesi F, Bertino EM, et al. ASCEND-7: efficacy and safety of ceritinib treatment in patients with ALK-positive non-small cell lung cancer metastatic to the brain and/or leptomeninges. Clin Cancer Res. 2022;28(12):2506–2516. doi: 10.1158/1078-0432.CCR-21-1838
60. Chalmers AW, Patel S, Boucher K, et al. Phase I trial of targeted EGFR or ALK therapy with ipilimumab in metastatic NSCLC with long-term follow-up. Target Oncol. 2019;14(4):417–421. doi: 10.1007/s11523-019-00658-0
61. Shaw AT, Lee S-H, Ramalingam SS, et al. Avelumab (anti–PD-L1) in combination with crizotinib or lorlatinib in patients with previously treated advanced NSCLC: Phase 1b results from JAVELIN Lung 101. J Clin Oncol. 2018;36(15_suppl):9008–. doi: 10.1200/JCO.2018.36.15_suppl.9008
62. Solomon BJ, Besse B, Bauer TM, et al. Lorlatinib in patients with ALK-positive non-small-cell lung cancer: results from a global phase 2 study. Lancet Oncol. 2018;19(12):1654–1667. doi: 10.1016/S1470-2045(18)30649-1
63. Ettinger DS, Wood DE, Aisner DL, et al. Non-small cell lung cancer, version 3.2022, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2022;20(5):497–530. doi: 10.6004/jnccn.2022.0025
64. West H, McCleod M, Hussein M, et al. Atezolizumab in combination with carboplatin plus nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol. 2019;20(7):924–937. doi: 10.1016/S1470-2045(19)30167-6
65. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N Engl J Med. 2018;378(24):2288–2301. doi: 10.1056/NEJMoa1716948
66. Socinski MA, Nishio M, Jotte RM, et al. IMpower150 final overall survival analyses for atezolizumab plus bevacizumab and chemotherapy in first-line metastatic nonsquamous NSCLC. J Thorac Oncol. 2021;16(11):1909–1924. doi: 10.1016/j.jtho.2021.07.009
67. Bylicki O, Tomasini P, Radj G, et al. Atezolizumab with or without bevacizumab and platinum-pemetrexed in patients with stage IIIB/

IV non-squamous non-small cell lung cancer with EGFR mutation, ALK rearrangement or ROS1 fusion progressing after targeted therapies: a multicentre phase II open-label non-randomised study GFPC 06-2018. Eur J Cancer. 2023;183:38–48. doi: 10.1016/j.ejca.2023.01.014

- **Atezolizumab plus bevacizumab and platinum-pemetrexed achieved promising efficacy in EGFR mutant or ALK/ROS1 rearranged NSCLC after TKI failure.**

68. Choudhury NJ, Young RJ, Sellitti M, et al. Lorlatinib and bevacizumab activity in ALK-Rearranged lung cancers after lorlatinib progression. JCO Precis Oncol. 2020;4(4):1333–1338. doi: 10.1200/PO.20.00271
69. Mota I, Patrucco E, Mastini C, et al. ALK peptide vaccination restores the immunogenicity of ALK-rearranged non-small cell lung cancer. Nat Cancer. 2023;4(7):1016–1035. doi: 10.1038/s43018-023-00591-2
70. Akbay EA, Koyama S, Carretero J, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov. 2013;3(12):1355–1363. doi: 10.1158/2159-8290.CD-13-0310
71. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443–2454. doi: 10.1056/NEJMoa1200690
72. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455–2465. doi: 10.1056/NEJMoa1200694
73. Rech AJ, Mick R, Martin S, et al. CD25 blockade depletes and selectively reprograms regulatory T cells in concert with immunotherapy in cancer patients. Sci Transl Med. 2012;4(134):134ra62. doi: 10.1126/scitranslmed.3003330
74. Rohaan MW, Borch TH, van den Berg JH, et al. Tumor-infiltrating lymphocyte therapy or ipilimumab in advanced melanoma. N Engl J Med. 2022;387(23):2113–2125. doi: 10.1056/NEJMoa2210233
75. Creelan BC, Wang C, Teer JK, et al. Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial. Nat Med. 2021;27(8):1410–1418. doi: 10.1038/s41591-021-01462-y
76. Huang J, Liu F, Li C, et al. Role of CD47 in tumor immunity: a potential target for combination therapy. Sci Rep. 2022;12(1). doi: 10.1038/s41598-022-13764-3
77. Maute R, Xu J, Weissman IL. CD47-SIRPα-targeted therapeutics: status and prospects. Immunooncol Technol. 2022;13:100070. doi: 10.1016/j.iotech.2022.100070
78. Champiat S, Cassier PA, Kotecki N, et al. Safety, pharmacokinetics, efficacy, and preliminary biomarker data of first-in-class BI 765063, a selective SIRPα inhibitor: results of monotherapy dose escalation in phase 1 study in patients with advanced solid tumors. J Clin Oncol. 2021;39(15_suppl):2623–. doi: 10.1200/JCO.2021.39.15_suppl.2623
79. Funazo TY, Ozasa H, Hashimoto K, et al. Abstract 1601: CD47 related to intratumor heterogeneity in alectinib-resistant ALK-rearranged lung cancer cell lines. Cancer Res. 2022;82(12_Supplement):1601–. doi: 10.1158/1538-7445.AM2022-1601
80. Vaccaro K, Allen J, Whitfield TW, et al. Targeted therapies prime oncogene-driven lung cancers for macrophage-mediated destruction. Preprint. bioRxiv. 2023. doi: 2023.03.03.531059.
81. Berraondo P, Sanmamed MF, Ochoa MC, et al. Cytokines in clinical cancer immunotherapy. Br J Cancer. 2019;120(1):6–15. doi: 10.1038/s41416-018-0328-y
82. Diab A, Tannir NM, Bentebibel SE, et al. Bempegaldesleukin (NKTR-214) plus nivolumab in patients with advanced solid tumors: phase i dose-escalation study of safety, efficacy, and immune activation (PIVOT-02). 2020;10(8):1158–1173. doi: 10.1158/2159-8290.CD-19-1510 *Cancer Discov*.
83. Harrington K, Freeman DJ, Kelly B, et al. Optimizing oncolytic virotherapy in cancer treatment. Nat Rev Drug Discov. 2019;18(9):689–706. doi: 10.1038/s41573-019-0029-0
84. Andtbacka RH, Kaufman HL, Collichio F, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol. 2015;33(25):2780–2788. doi: 10.1200/JCO.2014.58.3377
85. Kaufman HL, Kim DW, DeRaffele G, et al. Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma. Ann Surg Oncol. 2010;17(3):718–730. doi: 10.1245/s10434-009-0809-6
86. Gupta A, Probst HC, Vuong V, et al. Radiotherapy promotes tumor-specific effector CD8+ T cells via dendritic cell activation. J Immunol. 2012;189(2):558–566. doi: 10.4049/jimmunol.1200563
87. Vincent J, Mignot G, Chalmin F, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res. 2010;70(8):3052–3061. doi: 10.1158/0008-5472.CAN-09-3690
88. Slaney CY, Kershaw MH, Darcy PK. Trafficking of T cells into tumors. Cancer Res. 2014;74(24):7168–7174. doi: 10.1158/0008-5472.CAN-14-2458
89. Yeku OO, Purdon TJ, Koneru M, et al. Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment. Sci Rep. 2017;7(1):10541. doi: 10.1038/s41598-017-10940-8
90. Ye B, Stary CM, Li X, et al. Engineering chimeric antigen receptor-T cells for cancer treatment. Mol Cancer. 2018;17(1):32. doi: 10.1186/s12943-018-0814-0
91. Caruana I, Savoldo B, Hoyos V, et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. Nat Med. 2015;21(5):524–529. doi: 10.1038/nm.3833
92. Pilotto S, Molina-Vila MA, Karachaliou N, et al. Integrating the molecular background of targeted therapy and immunotherapy in lung cancer: a way to explore the impact of mutational landscape on tumor immunogenicity. Transl Lung Cancer Res. 2015;4(6):721–727. doi: 10.3978/j.issn.2218-6751.2015.10.11
93. Skoulidis F, Byers LA, Diao L, et al. Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. Cancer Discov. 2015;5(8):860–877. doi: 10.1158/2159-8290.CD-14-1236
94. Skoulidis F, Goldberg ME, Greenawalt DM, et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-Mutant lung adenocarcinoma. Cancer Discov. 2018;8(7):822–835. doi: 10.1158/2159-8290.CD-18-0099
95. Petroni G, Buqué A, Zitvogel L, et al. Immunomodulation by targeted anticancer agents. Cancer Cell. 2021;39(3):310–345. doi: 10.1016/j.ccell.2020.11.009