Review Article

Narrative review: immunotherapy in anaplastic lymphoma kinase (ALK)+ lung cancer—current status and future directions

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Background and Objective: Patients with metastatic anaplastic lymphoma kinase (ALK)+ non-small cell lung cancer (NSCLC) often experience years of disease control on targeted therapies but the disease eventually develops resistance and progresses. Multiple clinical trial efforts to incorporate PD-1/PD-L1 immunotherapy into the treatment paradigm for ALK+ NSCLC have resulted in significant toxicities without clear improvement in patient outcomes. Observations from clinical trials, translational studies, and preclinical models suggest the immune system interacts with ALK+ NSCLC and this interaction is heightened with the initiation of targeted therapy. The objective of this review is to summarize knowledge to date about current and potential immunotherapy approaches for patients with ALK+ NSCLC.

Methods: To identify the relevant literature and clinical trials the databases PubMed.gov and ClinicalTrials.gov were queried with keywords "ALK" and "lung cancer". PubMed search was further refined with terms such as "immunotherapy", "tumor microenvironment or TME", "PD-1", and "T cells". The search for clinical trials was limited to interventional studies.

Key Content and Findings: In this review, the current status of PD-1/PD-L1 immunotherapy for ALK+ NSCLC is updated and alternative immunotherapy approaches are highlighted in the context of available patient level and translational data on the ALK+ NSCLC tumor microenvironment (TME). An increase in $CD8^+$ T cells within the ALK+ NSCLC TME has been observed with targeted therapy initiation across multiple studies. Therapies to augment this including tumor infiltrating lymphocyte (TIL) therapy, modified cytokines, and oncolytic viruses are reviewed. Furthermore, the contribution of innate immune cells in TKI mediated tumor cell clearance is discussed as a future target for novel immunotherapy approaches that promote cancer cell phagocytosis.

Conclusions: Immune modulating strategies derived from current and evolving knowledge of the ALK+ NSCLC TME may have a role in ALK+ NSCLC beyond PD-1/PD-L1 based immunotherapy.

Keywords: Anaplastic lymphoma kinase (ALK); non-small cell lung cancer (NSCLC); tumor microenvironment (TME); immunotherapy

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Introduction

Approximately 4–5% of lung cancers are driven by an oncogenic anaplastic lymphoma kinase (ALK) fusion event (1-4). Patients with ALK+ non-small cell lung cancer (NSCLC) often experience years of disease control with the current generation of ALK specific tyrosine kinase inhibitors (TKI) (5-7). However, even with these highly effective therapies, persistent disease remains and resistance to targeted therapy will eventually develop requiring a therapy change to an alternative TKI or a chemotherapy based regimen (8). In parallel to the development of more effective TKI therapy for patients with ALK+ NSCLC, immunotherapy through blockade of programmed cell death protein 1 (PD-1) or programmed cell death 1 ligand

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Table 1 The search strategy summary: PubMed

Items	Specification
Date of search	December 1, 2022
Databases and other sources searched	PubMed
Search terms used	Anaplastic lymphoma kinase/ALK AND lung cancer/NSCLC, AND/OR PD-1, immune system, immunotherapy, T cell, tumor microenvironment, TME
Timeframe	All time
Inclusion and exclusion criteria	Language: English
Selection process	ELS conducted the literature search and data selection

ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1, TME, tumor microenvironmen.

Table 2 The search strategy summary: ClinicalTrials.gov

Items	Specification
Date of search	December 1, 2022
Databases and other sources searched	ClinicalTrials.gov
Search terms used	Condition or disease: Lung Cancer, Other terms: ALK
Timeframe/status	Recruiting and not yet recruiting studies
Inclusion and exclusion criteria	Study type: interventional
Selection process	ELS conducted the literature search and trial selection
Any additional considerations, if applicable	Trials were reviewed for the use or application of immune modulating or immunotherapy based approaches

ALK, anaplastic lymphoma kinase.

1 (PD-L1) redefined the standard of care for most patients with lung cancer (8). For patients with early stage disease, immunotherapy can increase the chance at long term survival through neoadjuvant, adjuvant, or consolidative approaches (9-11). Remarkably, for patients with metastatic disease treated with an immunotherapy based regimen, long term survival is also a possibility for a subset of patients with a predefined duration of therapy (12-14). Currently, immunotherapy with PD-1 or PD-L1 based immunotherapy does not have a clear role in the treatment of patients with ALK+ NSCLC and novel approaches to augment the anti-tumor immune response in these patients is an area of unmet clinical need.

This narrative review summarizes the preclinical and translational data that lead to initial enthusiasm for the clinical trials incorporating PD-1 or PD-L1 based immunotherapy into the treatment paradigm for patients with ALK+ NSCLC. Furthermore, this work highlights preclinical and translational data that identify ways in which the immune system recognizes ALK+ NSCLC and how the ALK+ NSCLC tumor microenvironment (TME) is altered with TKI initiation. Future clinical trials and novel immunotherapy approaches are discussed in the context of the current knowledge of the ALK+ NSCLC TME. I present the following article in accordance with the Narrative Review reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-883/rc)

Methods

Relevant literature for this narrative review was obtained via searches of online databases including PubMed and ClinicalTrials.gov. PubMed was queried using key word "anaplastic lymphoma kinase" or "ALK" and "lung cancer", along with other terms as highlighted in *Table 1* and *Table 2*. A search was conducted to include relevant publications from time to inception of the database to December 2022 and was restricted to publications in

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Table 3 Selected studies of ALK+ NSCLC and PD-L1 expression

Total patient samples	PD-L1 detection method	Scoring method	Number of samples PD-L1 positive	Reference
10	PD-L1 antibody ab58810 (Abcam)	Staining intensity: 0 negative or trace, 1 weak, 2 moderate and 3 high. Positive samples contained a staining intensity ≥2 in more than 5% of tumor cells	6	(15)
19	PD-L1 antibody (clone E1L3N, Cell Signaling Technology)	PD-L1 positivity: membranous ± cytoplasmic staining of tumor cells of any intensity using cutoffs of ≥1%, ≥5%, and ≥50% tumor cells	PD-L1 ≥5%: 9; PD-L1 ≥50%: 5	(1)
9	22C3 pharmDx assay (Agilent Technologies)	PD-L1 tumor proportion score was calculated as the percentage of at least 100 viable tumor cells with complete or partial membrane staining	PD-L1 TPS 1–49%: 3; PD-L1 TPS ≥50%: 2	(16)

ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death 1 ligand 1; TPS, tumor proportion score.

English. Additional eligible studies were identified through manual review and searching of the reference list of included studies. ClinicalTrials.gov was queried in December 2022 using condition or disease: "Lung Cancer" and other terms "ALK". Recruitment status was set to "not yet recruiting" or "recruiting" and study type as "interventional (clinical trial)".

Preclinical data

ALK+ NSCLC expresses PD-L1

As data were emerging that PD-L1 expression associated with PD-1 immunotherapy response, initial investigations reported PD-L1 positivity in tumor specimens from patients with ALK+ NSCLC (*Table 3*). Using a semiquantitative immunohistochemistry (IHC) scoring method, moderate or high staining intensity of PD-L1 in ≥5% of tumor cells was considered positive, and 6 of 10 ALK+ NSCLC specimens were reported positive for PD-L1 (15). In a cohort of 19 patients with ALK+ NSCLC, PD-L1 expression (≥5% staining of tumor cells of any intensity) was positive in 9 patients prior to TKI therapy, and 5 of those patients had tumors with PD-L1 positivity in ≥50% of tumor cells (16). While early reports used a variety of methodologies and definitions of positivity, PD-L1 assessment by IHC with 22C3, a companion diagnostic assay for pembrolizumab, found 5 of 9 tumor samples from patients with ALK+ NSCLC expressed PD-L1 and 2 samples demonstrated PD-L1 ≥50% (17,18).

PD-L1 expression associates with oncogenic ALK fusion signaling

In a series of studies, ALK+ NSCLC PD-L1 expression was connected to the activation of multiple canonical signaling pathways downstream of oncogenic ALK fusions (19). Patient derived NSCLC cell lines bearing an ALK fusion were found to express PD-L1 at baseline and inhibition of the ALK fusion protein through RNA silencing or ALK-targeting TKIs downregulated PD-L1 cell surface expression (20). Pharmacologic inhibition of the phosphoinositide 3-kinases (PI3K) pathway and the mitogen-activated protein kinases (MAPK) pathway through MEK inhibition resulted in decreased cell surface PD-L1 expression, suggesting these pathways downstream of ALK fusion proteins regulate PD-L1 expression (20). Signaling through signal transducer and activator of transcription 3 (STAT3) and hypoxia inducible factor 1α (HIF1α) were associated with PD-L1 expression in ALK+ NSCLC cell lines and, in tissue from patients with ALK+ NSCLC, expression of either hosphor-STAT3 or HIF1α by IHC associated with tumor PD-L1 expression (21). Inhibition of yes association protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ), components of the Hippo pathway, reduced ALK fusion driven PD-L1 upregulation (22).

These observations from patient specimens and *in vitro* studies that ALK+ NSCLC expressed PD-L1 and was driven by oncogenic ALK fusion protein signaling strengthened the concept of ALK-mediated local immunosuppression

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via PD-L1 expression. Experiments with *ex vivo* activated dendritic cells and autologous cytokine-induced killer cells (DC-CIK) showed increased ALK+ NSCLC cell line sensitivity to cell-mediated cytotoxicity when cell lines were cultured in the presence of DC-CIK and anti-PD-1 (23). In total, these results supported the rationale for incorporating immunotherapy into the treatment paradigm for patients with ALK+ NSCLC.

Clinical trial data

Single agent immunotherapy in ALK+ NSCLC

Early studies that established the role of PD-1 or PD-L1 immunotherapy in subsequent line settings for metastatic NSCLC included patients with ALK+ NSCLC. In each of these prospective randomized studies, immunotherapy, whether nivolumab (CheckMate 057), pembrolizumab (KEYNOTE-010), or atezolizumab (POPLAR and OAK), demonstrated superior overall survival (OS) compared to docetaxel in the intention to treat population (24-27). Only CheckMate 057 and KEYNOTE-010 enrolled patients with ALK+ NSCLC into both the immunotherapy arm and docetaxel arm while the studies with atezolizumab did not (24-27). Due to the small number of patients with ALK+ NSCLC enrolled onto the CheckMate and KEYNOTE studies, subgroup analyses for ALK+ NSLC were not reported. However, initial clues to the level of efficacy were found in the subgroup of patients with NSCLC bearing epidermal growth factor receptor (EGFR) mutation. Unlike the overall trial population, patients with EGFR+ NSCLC did not experience benefit with either nivolumab (OS HR 1.18, 95% CI: 0.69–2.00), pembrolizumab (OS HR 0.88, 95% CI: 0.45–1.70) or atezolizumab (OS HR 1.24, 95% CI: 0.71–2.18) in the subsequent line setting (24,25,27).

Single arm and retrospective studies further reinforced the limited efficacy of single agent PD-1 or PD-L1 inhibition in ALK+ NSCLC. The ATLANTIC trial was a single arm, phase 2 study testing durvalumab in the third line or later setting for metastatic NSCLC, including patients with ALK+ NSCLC (28). None of the 11 evaluable patients with ALK+ NSCLC experienced a confirmed disease response (28). A retrospective review from a single institution in the United States identified 6 patients with ALK+ NSCLC who received PD-1 or PD-L1 immunotherapy and none were found to have a radiographic response (16). Similarly, a combined report from an institution in the United States and in China included 13 patients with ALK+ NSCLC who received PD-1 or PD-L1 immunotherapy, and none of the patients demonstrated a radiographic response (29). The international, multi-institution IMMUNOTARGET registry assessed the response of oncogene-driven lung cancer to PD-1 or PD-L1 immunotherapy and included 23 patients with ALK+ NSCLC (30). In the 19 patients with ALK+ NSCLC and available response data, none experienced disease response (30). A retrospective review of 14 patients with ALK+ NSCLC treated in South Korea reported 2 patients with partial responses to pembrolizumab, one for 8.2 months and the other with an ongoing response at 4.1 months (31). In a cohort of 8 patients with ALK+ NSCLC at institutions across France, 2 patients experienced a partial response to PD-1 or PD-L1 immunotherapy (32). With longitudinal electronic health record data from hundreds of cancer clinics within the United States, investigators identified 83 patients with ALK+ NSCLC who received PD-1 or PD-L1 immunotherapy (33). In this cohort, the median time to progression was 2.34 months (95% CI: 1.55–3.09) (33).

Two case reports describe patients with ALK+ NSCLC who experienced disease response on PD-1 immunotherapy after exhausting available TKI options. After 2 TKIs and platinum doublet chemotherapy, a patient with an ALK G1202R mutation identified by next generation sequencing (NGS) received pembrolizumab and experienced a partial response through 9 cycles (34). A second patient with ALK+ NSCLC, received ceritinib then a platinum doublet and bevacizumab prior to progression (35). NSG of a progressive lesion found an ALK rearrangement and no apparent resistance mechanisms. The patient received 3rd line nivolumab and a complete response was noted on imaging for approximately 16 months (35).

Taken together, of the 73 patients with ALK+ NSCLC who received PD-1 or PD-L1 immunotherapy reported in the literature, only 6 experienced disease response, for an overall response rate of 8%.

Immunotherapy plus TKI in ALK+ NSCLC

Prior to the readout of several early phase clinical trials, the combination of TKI and immunotherapy was a promising avenue for patients with ALK+ NSCLC. With the non-overlapping mechanisms of actions, these therapies had the potential to increase responsiveness to TKI and, given overall tolerability of both therapies, the hope was additive toxicities would be limited. Unfortunately, neither of these

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assumptions held.

The combination of crizotinib and nivolumab was tested in CheckMate 370 and stopped early due to severe adverse events (36). Thirteen patients with ALK+ NSCLC starting first line crizotinib were enrolled, and 5 experienced grade ≥3 hepatic toxicities (36). Two patients died within months of starting the combination therapy with acute hepatic toxicities as part of their presentation prior to death (36) A partial response was observed in 38% of patients, a rate markedly less than what would be expected with crizotinib in the first line setting (37). Nivolumab and ceritinib in the first line and the subsequent line setting resulted in a 69% and a 35% response rate, respectively, both similar to expectations of ceritinib monotherapy (38-40). Six dose limiting toxicities (DLTs) were due to gastrointestinal adverse events including pancreatitis, autoimmune hepatitis, elevated lipase, and elevated transaminase (38). Gastrointestinal toxicities were the main DLTs observed in the combination of crizotinib and pembrolizumab (41). Nine treatment-naïve patients with ALK+ NSCLC were enrolled and 4 experienced a DLT including a pembrolizumab related pneumonitis that contributed to a patient's death (41). Five patients experienced a disease response, but due to toxicity the study was terminated early (41).

Ipilimumab, an immunotherapy that blocks cytotoxic T-lymphocyte-associated protein 4, was tested in patients with EGFR+ or ALK+ NSCLC CTLA-4 who were on stable dose of oncogene-targeting TKI (42). Three patients with ALK+ NSCLC receiving crizotinib were enrolled on the trial and one developed grade 3 hypophysitis and another patient experienced grade 2 pneumonitis (42). The trial was stopped early due to toxicities in observed in both patient cohorts and response rate was not reported (42).

Based on a limited number of observations later generations of ALK-directed TKIs appeared to have less toxicity when combined with immunotherapy but no clear signal of improved efficacy. JAVELIN Lung 101 combined avelumab with lorlatinib in 28 patients with previously treated ALK+ NSCLC (43). No DLTs occurred, grade ≥3 gamma-glutamyl transferase elevation was observed in 2 patients, and response rate was 46.4% in line with expectations for subsequent line lorlatinib alone (43,44). Twenty-one treatment naive patients with ALK+ NSCLC received alectinib alone for 7 days and then started atezolizumab (45). No DLTs or grade 4 or 5 adverse events were reported (45). Response was observed in 81% of patients, similar to first line alectinib monotherapy (46).

Immunotherapy plus chemotherapy in metastatic ALK+ NSCLC

Immunotherapy plus chemotherapy is the standard of care for most patients with metastatic NSCLC (8). For most of the clinical trials that established these options, patients with ALK+ NSCLC were excluded. Two trials testing the incorporation of atezolizumab into chemotherapy with or without bevacizumab allowed enrollment of patients with EGFR+ or ALK+ NSCLC after progression or intolerance to at least 1 TkI.

IMpower130 compared the combination of carboplatin plus nab-paclitaxel with and without atezolizumab (47). IMpower150 tested atezolizumab plus carboplatin plus paclitaxel (ACP), bevacizumab plus carboplatin plus paclitaxel (BCP), or atezolizumab plus BCP (ABCP) (48). Both trials met their primary endpoint of improved OS with the incorporation of atezolizumab to either carboplatin plus nab-paclitaxel or BCP. IMpower130 enrolled 32 patients with EGFR+ or ALK+ NSCLC onto the atezolizumab + chemotherapy arm and 12 onto the chemotherapy alone arm. No benefit in OS or progression free survival (PFS) was observed in this subgroup analysis (47). On IMpower150, 44 patients with EGFR+ or ALK+ NSCLC received ABCP and 64 received BCP (48). Subgroup analysis of patients with EGFR+ or ALK+ NSCLC was positive for PFS (HR 0.59; 95% CI: 0.37–0.94) and OS (HR 0.61; 95% CI: 0.52–0.72) for patients treated with ABCP *vs.* BCP (48). Whether this improvement in overall survival is biologically relevant or due to a small sample size that is unbalanced remains unclear.

Curative intent immunotherapy in early stage ALK+ NSCLC

As immunotherapy moved to earlier stages of NSCLC, patients with EGFR+ or ALK+ NSCLC were variably included. The PACIFIC trial demonstrated that 1 year of durvalumab consolidation after concurrent chemoradiation significantly improved PFS and OS for patients with locally advanced, unresectable NSCLC (49,50). Eight patients with ALK+ NSCLC were enrolled onto the trial and were grouped with 27 patients with EGFR+ NSCLC for outcome analyses (11). No difference in OS was observed in the EGFR+ or ALK+ NSCLC subgroup comparing durvalumab consolidation to placebo (HR 0.85, 0.37–1.97) (11). Adjuvant atezolizumab after chemotherapy is standard of care for patients with resected NSCLC with PD-L1 ≥1% based on

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the IMpower010 trial (10). Patients with ALK+ NSCLC were allowed to enroll and 12 were randomized to the immunotherapy arm and 11 received best supportive care (10). No disease free survival benefit was observed with adjuvant atezolizumab in patients with ALK+ NSCLC (HR 1.05; 95% CI: 0.32–3.45) (10). Patients with ALK+ NSCLC were excluded from CheckMate 816, a phase 3 clinical trial that demonstrated improved event free survival with neoadjuvant nivolumab plus platinum doublet chemotherapy compared to chemotherapy alone (9).

The available clinical trial and retrospective data suggest that the clinically available immunotherapies for lung cancer have limited to no role in the treatment of patients with ALK+ NSCLC. While some may broadly interpret this body of knowledge to suggest the immune system has no role in the control of ALK+ NSCLC, an alternative hypothesis is that the optimal immunotherapy intervention for patients with ALK+ NSCLC lies beyond PD-1 or PD-L1. A growing body of literature suggests the immune system does interface with ALK+ NSCLC and response to TKI therapy.

The ALK+ NSCLC interface with the immune system

Wild type ALK protein expression is primarily within the nervous system and through various knockout models, including mice, it is believed to contribute to embryonic neural development, neurogenesis, and behavioral regulation (51-55). Studies of ALK protein expression in human tissue have demonstrated high levels of expression within structures of the brain with low levels of expression in the testis and colon (56-58). Based on the likely limited exposure of the wild type ALK protein to the immune system due to its developmental localization within the nervous system, oncogenic ALK fusions may be antigenic to the immune system.

Immune system recognition of ALK+ NSCLC

Translational studies from patients with ALK+ malignancies suggest an adaptive immune response can be generated *de novo* against oncogenic ALK fusions. The presence of antibodies against oncogenic ALK fusions was tested for in 21 patients with metastatic ALK+ NSCLC (59). At the time of testing, two patients were treatment naïve, one was receiving pemetrexed chemotherapy, and 18 were on an ALK-targeting TKI (59). Thirteen of these patients were positive for anti-ALK fusion antibodies and, in comparison none of the 20 healthy controls had detectable anti-ALK fusion antibodies (59). In a series of 53 patients with metastatic ALK+ NSCLC, 9 patients were positive for a high level of anti-ALK antibodies when compared to 38 patients with ALK negative NSCLC (60). In the 9 patients with high anti-ALK antibodies, epitope mapping revealed antibody recognition predominantly in the cytoplasmic domain (60).

T cell responses have been observed in animal models of ALK+ NSCLC. In an orthotopic mouse model of ALK+ NSCLC, animals vaccinated with a DNA plasmid coding for the intracytoplasmic domain on ALK developed significantly fewer tumor nodules compared to animals that received a control vaccination (61). This vaccine strategy also decreased tumor out growth and increased survival in animals transgenic for ALK under the control of the lung specific surfactant protein promoter (61). In both the orthotopic and transgenic models of ALK+ NSCLC, the ALK vaccine generated ALK specific T cell immunity (61). In the ALK transgenic animals, and tumor control associated with increased numbers of $CD8^+$ T cells within the tumors and was dependent on the presence of CD8+ T cells (61). In patients with anaplastic large cell lymphomas, $CD8^+$ and $CD4^+$ T cell responses have been detected against peptides derived from oncogenic ALK fusions, though these have not yet been reported in patients with ALK+ NSCLC (62-67).

The ALK+ NSCLC TME

While data suggest the oncogenic ALK fusion in ALK+ NSCLC can be immunogenic, studies of the ALK+ TME have revealed an immunologically cold TME. In a cohort of 6 patients with ALK+ NSCLC, IHC for CD3+, CD4+, and CD8+ cells revealed significantly reduced levels of T cells within the ALK+ TME compared to the EGFR+ NSCLC TME (61). Gene set enrichment analysis of a publically available data set of resected, early stage NSCLC that included 11 patients with ALK+ NSCLC, 127 with EGFR+ NSCLC, and 20 with Kristen rat sarcoma virus (KRAS) mutation positive NSCLC, demonstrated that the transcriptional profile of the ALK+ NSCLC TME contained fewer T cell related transcripts compared to the other patient cohorts (61,68). In 13 treatment naïve patients with ALK+ NSCLC, 10 of the ALK+ TME were found to have negative to low infiltration of CD8+ cells and 3 contained a moderate level of infiltration by IHC (16). In a

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separate cohort, 9 patients with crizotinib resistant ALK+ NSCLC all were found to have negative to low infiltration of $CD8^+$ cells (16).

Within the TME of ALK+ NSCLC, infiltrating immune cells are reported to vary across studies but in general, exhibit reduced levels of activity. Gene set enrichment analysis of bulk RNA sequencing from 14 patients with ALK+ NSCLC revealed a diminished interferon-γ-related response signature when compared to ALK– NSCLC (31). In 25 treatment naïve ALK+ NSCLC patients, $CD8^+$ T cell infiltration was noted within the tumor compartment and stroma at levels equivalent to or moderately reduced when compared to controls, respectively (69). A major difference was noted in levels of interferon-γ transcript within the $CD8^+$ T cells where control tumors contained $CD8^+$ T cells that were positive for interferon-γ mRNA, but in ALK+ NSCLC $CD8^+$ T cells were negative for interferon-γ mRNA suggesting an ineffective effector T cell response (69). In comparison to KRAS mutation positive NSCLC, IHC of tumor specimens from 39 patients with ALK+ NSCLC demonstrated fewer infiltrating $CD3^+$ and $CD8^+$ cells and reduced levels of granzyme B, which may suggest reduced T cell activity but could reflect reduced T cell numbers overall (70). Comparative mRNA studies of 31 samples from ALK+ NSCLC patients who were treatment naïve found an increase in T regulatory (T_{reg}) cells within the ALK+ NSCLC TME compared to patients with EGFR+ NSCLC or patients with ALK– and EGFR– NSCLC (71). However, this difference in T_{reg} cells was not seen by IHC in a cohort of 39 patients with ALK+ NSCLC (70). Within the cohort of 39 patients with ALK+ NSCLC, $CD8^+$ T cell expression of checkpoints PD-1, CTLA-4, lymphocyte activating gene 3 (Lag3), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) by IHC was markedly reduced (70). This suggests infiltrating $CD8^+$ T cells were unable to engage with potential tumor antigens as T cell checkpoint expression is upregulated by T cell activation (72).

Turning the ALK+ NSCLC from cold to hot: a new benefit from an old friend?

A major theme of current cancer drug development, in general, is to increase T cell infiltration and activation within the TME as T cells are key to tumor control and clearance (73). One route is to induce immunogenic cell death (ICD) within the cancer cell to activate the adaptive immune system. ICD results in the heightened recognition of cancer cells by innate immune cells such as dendritic cells and macrophages and promotes their differentiation into activated phenotypes which can recruit and stimulate a T cell response (74). Crizotinib was found to induce multiple markers of ICD in H2228, a patient-derived ALK+ NSCLC cell line (75). Ceritinib induced ICD in H3122, a patient derived ALK+ NSCLC cell line, via pyroptosis (76). The induction of ICD by TKI may alter the TME in ALK+ NSCLC to a more immunologically active environment and several lines of evidence from patient data and mouse models support this.

The first patient level data was generated by the phase Ib clinical trial of alectinib plus atezolizumab on which treatment naïve ALK+ NSCLC patients received 7 days of alectinib prior to atezolizumab (45). On treatment biopsies were obtained for 9 patients after the alectinib run in and before the initiation of atezolizumab. When compared to pre-treatment biopsies, an increase in $CD8^+$ T cell infiltration was observed in 7 out of 9 patient samples by IHC (45). Bulk RNA sequencing was performed on pre-treatment tissue samples and samples at time of response or progression on TKI from 8 patients with ALK+ NSCLC in addition to 23 patients with EGFR+ NSCLC (77). In response to TKI treatment, significant upregulation was noted in genes supporting T cell activation and differentiation in both patient cohorts (77). Using bioinformatics approaches to deconvolute the data and identify immune cell subsets, findings from the ALK samples showed an increase in TME immune score with TKI treatment and an increase in cytotoxic cells which comprises $CD8^+$ T cells, gamma-delta T cells and natural killer (NK) cells within the TME (77). Similarly, single cell RNA sequencing and multispectral tissue imaging demonstrated an increase in TME T cell infiltration a few weeks after TKI initiation in a cohort of 30 patients starting TKI, including 10 patients with ALK+ NSCLC (78).

In two different animal models of ALK+ NSCLC, parallels have emerged with the patient data. Mice transgenic for an EML4-ALK fusion under a lung specific promoter were treated with ceritinib until progression on imaging (79). Flow cytometry on progressing tumors revealed an increase in T_{reg} cells, a cell population that was increased at time of progression in patients receiving a range of TKIs (78,79). A significant influx of $CD3^+CD8^+$ cells was found 4 days after TKI initiation in an orthotopic model of EML4-ALK+ NSCLC that experiences complete response to TKI (80). In contrast, a distinct orthotopic model that only experiences a partial response to TKI showed a limited

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increase in $CD3^+CD8^+$ cell TME infiltration (80). For both orthotopic models, response to TKI was dependent on a functional adaptive immune system, as tumor control on TKI occurred for a limited time and was not durable in nude mice (80).

Taken together, these data from patients with ALK+ NSCLC and murine models suggests T cell TME infiltration occurs with TKI initiation and may influence response to TKI. While some patients with ALK+ NSCLC experience a robust, long-term response to TKI therapy, there is heterogeneity across patients with ALK+ NSCLC in the depth and duration of response to therapy. One possibility is that the immune composition of the TME, shaped by both the cancer and its response to TKI, can influence the heterogeneity of these outcomes. Beyond the immediate focus on the T cell compartment, innate immune cells within the TME can impact not only T cell activation but also may have direct interactions with ALK+ NSCLC. Macrophage conditioned media imparted alectinib resistance through MET bypass signaling in an *in vitro* model system with EML4-ALK+ NSCLC cells derived from murine models (81). Furthermore, H3122 xenografts demonstrated improved control with crizotinib when animals were also treated with clodronate, an agent that broadly depletes macrophages (81). CD47 is a widely expressed cell surface molecule that prevents target cell phagocytosis by innate immune cells through its interaction with signal regulatory protein alpha (SIRPα) and CD47 has been found to be overexpressed in many cancer types, including lung cancer (82). *In vitro* establishment of alectinib resistant H2228 was found to generate two distinct cell populations based on CD47 expression (83). Establishment of the $CD47^{Hi}$H2228 or $CD47^{Lo}$H2228 in nude mice revealed the significant tumorgenicity of the $CD47^{Hi}$ subpopulation and marked outgrowth (82). Anti-CD47 therapy improved the degree of tumor control with TKI in an immunodeficient mouse model (84). In a separate study, animals bearing H3122 tumors were treated with lorlatinib, anti-CD47 or the combination (84). Animals that received combination therapy experienced the most pronounced tumor shrinkage and duration of response (84).

Clinical trials and future approaches incorporating immunotherapy for ALK+ NSCLC

Current trials

Following up on the efficacy signal from IMpower150, several clinical trials are underway to test whether the incorporation of PD-1 or PD-L1 directed immunotherapy into chemotherapy with and without anti-angiogenic therapy is beneficial for patients with ALK+ NSCLC after progressing on TKI (*Table 4*).

One randomized phase III trial and one large phase II study are recruiting patients with ALK+ NSCLC to test atezolizumab in combination with a platinum doublet with or without bevacizumab in the setting of TKI progression (85,86). A small phase II study anticipated to enroll will evaluate pembrolizumab with bevacizumab and chemotherapy in patients with ALK+ NSCLC after progression on first line alectinib (87). A fourth study is planned using camrelizumab, a PD-1 inhibitor, with apatinib, a small molecule inhibitor of vascular endothelial growth factor receptor 2, in combination with carboplatin and pemetrexed (88).

Adoptive cell therapy with tumor infiltrating lymphocytes (TILs) represents a new avenue for immunotherapy in solid malignancies, including lung cancer. In a phase I clinical trial of TILs given with nivolumab in patients with metastatic NSCLC, 3 patients who received TILs and nivolumab experienced disease response, including a complete response in a patient with EGFR+ NSCLC who progressed on osimertinib (89). $CD8^+$ and $CD4^+$ TILs generated from the patient with an EGFR+ NSCLC were found to be reactive to multiple epitopes from several cancer testis antigens and one somatic mutation (89). This patient had limited positivity for established and experimental biomarkers of immunotherapy response. Tumor PD-L1 was 2% and estimates of the patient's tumor mutational burden were low based on commercial testing and whole exome sequencing (89). A large, multi-cohort phase II trial is ongoing evaluating TIL therapy with and without checkpoint immunotherapy, including cohorts that allow the enrollment of patients with ALK+ NSCLC (*Table 4*) (90). Other adoptive cell therapies under investigation include autologous NK cell therapy in combination with chemotherapy with and without cetuximab (91). This phase I/IIa study is only enrolling patients with lung cancer post-TKI progression, including ALK+ NSCLC (91).

Future directions

While the current clinically available checkpoint inhibitor immunotherapies have yet to find a clear place in the treatment paradigm for patients with ALK+ NSCLC, other immune modulating approaches may better support TKI-

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Table 4 Select current clinical trials for ALK+ NSCLC incorporating immunotherapy

Regimen	Study design	NCT number	Primary outcome	Estimated completion date
Carboplatin + pemetrexed + atezolizumab + bevacizumab *vs.* Carboplatin + pemetrexed + atezolizumab	Non-randomized phase II	NCT04042558	ORR	June 2024
Carboplatin + pemetrexed + atezolizumab + bevacizumab *vs.* carboplatin + pemetrexed	Randomized phase III	NCT03991403	PFS	December 2022
Pembrolizumab + chemotherapy + bevacizumab	Single arm phase II	NCT05266846	PFS	February 2024
Camrelizumab + apatinib meylate + pemetrexed + carboplatin	Single arm phase II	NCT04425135	ORR	January 2025
Autologous TIL (LN-145) +/– pembrolizumab	Phase II	NCT03645928	ORR, TEAE	December 2024
SNK01 (Super Natural Killer Cells 01) + gemcitabine + carboplatin +/– cetuximab	Phase I/IIa	NCT04872634	MTD, AE	May 2023

ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer; NCT, national clinical trials; ORR, overall response rate; PFS, progression free survival; TEAE, treatment emergent adverse event; MTD, maximum tolerated dose; AE, adverse event.

mediated tumor clearance and the endogenous immune response against ALK+ NSCLC (*Figure 1*). Recent advances in the engineering of cytokines have generated second-generation compounds that better enhance adaptive immune responses (92). Modified versions of IL-2, a major driver of T cell response, are able to bind with high affinity to effector T cells and are unable to interact with the IL-2 receptor present on T_{reg} cells (92). Modified IL-2 may promote the acquisition of effector functions of T cells already present in the ALK+ NSCLC TME while avoiding driving proliferation and function of T_{reg} cells that have also been identified in the ALK+ NSCLC TME (16,45,61,68-71,77,78). A phase I study of modified IL-2 plus nivolumab, including 5 patients with treatment-naïve, metastatic NSCLC, showed an increase in TME $CD8^+$ T cells on treatment and no increase in T_{reg} cells (93).

Oncolytic virus therapy is another potential tool to augment the immunogenicity of ALK+ NSCLC. These viral agents selective replicate within tumor cells causing immunogenic cell death (94). Notably, these viral agents can readily be modified to express cytokines or chemokines to improve antitumor efficacy (94). This strategy is already clinically available for patients with melanoma who can receive intralesional talimogene laherperepvec (T-VEC), a modified herpes virus that also expresses granulocyte macrophage colony-stimulating factor to recruit and activate antigen presenting cells (95). Translational studies demonstrated lesions injected with T-VEC contained more tumor antigen specific T cells with a decrease in T_{reg} and myeloid derived suppressor cells (96). While intralesional injections may be possible at time of maximal response on an ALK-targeting TKI, or at time of oligoprogression, systemic delivery of oncolytic virus therapy through intravenous infusion remains an attractive but elusive goal (94).

Finally, while the majority of immune modulating therapies are directed towards T cells, a new generation of immunotherapies for cancer targeting innate immune cells is starting to emerge (97). Macrophages, mononuclear phagocytes which can occupy functional states ranging from pro-immunogenic to pro-tumorigenic, can recognize and phagocytose cancer cells, influence cancer cell response to therapy, TME features including T cell infiltration and metabolic niche, and the tumor metastatic potential (97). The function of cancer cell recognition and phagocytosis by macrophages is, in part, influenced by the expression of CD47 on the cancer cells, which has been shown to be overexpressed in NSCLC (98). Clinical development of anti-CD47 and anti- SIRPα therapies, alone and in combination approaches, is furthest along in hematological malignancies (99). Data from early phase clinical trials suggests monotherapy has limited efficacy with more

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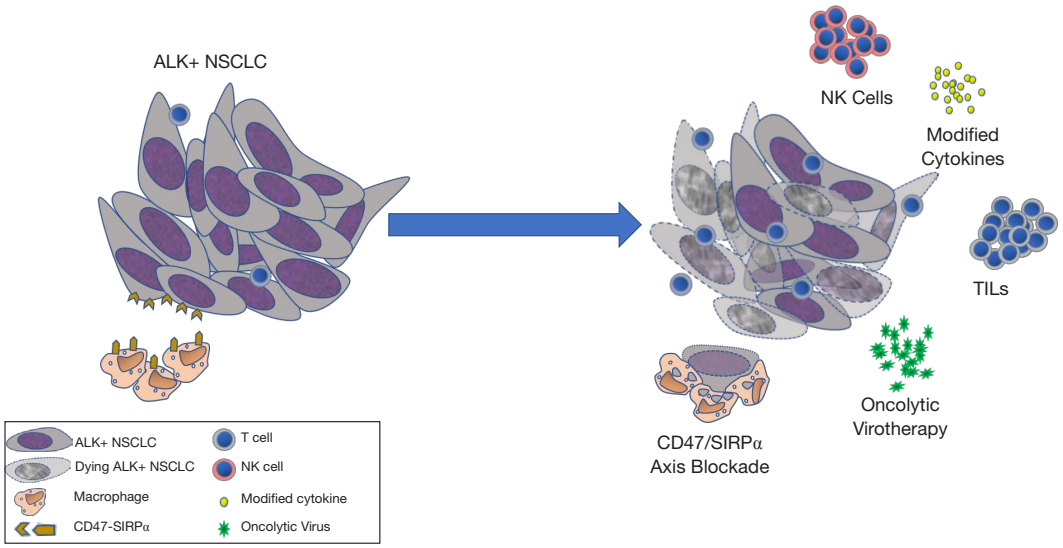

Figure 1 Future immune based approaches to augment the tyrosine kinase inhibitor response in ALK+ NSCLC. Schematic diagram of tyrosine kinase inhibitor mediated ALK+ NSCLC cell death and changes to the T cell content of the tumor microenvironment. Potential strategies to amplify the immune response to ALK+ NSCLC cell death include adoptive cell therapy with NK cells or tumor infiltrating lymphocyte therapy, modified cytokines that preferentially target effector cells, oncolytic virus therapy to increase tumor cell death and inflammatory milieu within the tumor microenvironment, and novel immune checkpoints that enhance innate immune cell recognition of ALK+ NSCLC cells. ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer; NK, natural killer; TILs, tumor infiltrating lymphocytes.

response observed when used in combination (99). To date, 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 (99,100). Based on clinical and preclinical data, this suggests the potential of an ALK-targeting TKI in combination with CD47/SIRPα axis directed therapies as an immunotherapy approach for ALK+ NSCLC.

Conclusions

The promise of immunotherapy has yet to reach patients with ALK+ NSCLC. Based on pre-clinical and translational data, the immune system in patients with ALK+ NSCLC recognizes and has a degree of interface with the cancer cells. While this interface has not yet been augmented by the current clinically available immune checkpoint inhibitors, promise remains beyond the PD-1/PD-L1 axis. Future efforts to identify effective immune modulating therapies for ALK+ NSCLC will require a better understanding of how TKI therapy alters the immune recognition of ALK+ NSCLC, how residual disease avoids immune detection, and which infiltrating immune cells are key to improving TKI response and patient outcomes.

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