Immunotherapy after EGFR-TKI treatment in advanced non-small cell lung cancer: Current status and future perspectives (Review)

HUIYUAN MA[1,2*], LONGHUI LI[2,3*], CONGHAN JIAO[1,2], YANYAN CHENG[1,2], JIAYU HE[1,2], CHEN JIANG[1,2], QIAN TONG[1,2], DAN YI[2,4] and YING ZHANG[1,2]

[1]Department of Hematology, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300193, P.R. China; [2]National Clinical Research Center for Chinese Medicine, Tianjin 300193, P.R. China; [3]Department of Acupuncture and Moxibustion, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300193, P.R. China; [4]Department of Oncology, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300193, P.R. China

Received June 9, 2025; Accepted January 2, 2026

DOI: 10.3892/or.2026.9049

Abstract. The tumor microenvironment (TME) of epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC) exhibits notable immunosuppressive properties. EGFR tyrosine kinase inhibitors (EGFR-TKIs) induce dynamic remodeling of the TME. By boosting the infiltration of immune cells such as T cells and dendritic cells and decreasing immunosuppressive elements such as tumor-associated macrophages and regulatory T cells, short-term TKI treatment can effectively enhance antitumor immunity. However, the TME changes to an immunosuppressive state marked by PD-L1 upregulation and immune escape with continued therapy and the emergence of resistance. This creates a transient immunotherapy window period during EGFR-TKI treatment, when immune checkpoint inhibitors may achieve optimal efficacy. It is essential to identify and take advantage of this window in order to enhance treatment results. The present review highlights the importance of understanding TME dynamics in EGFR-mutant NSCLC to optimize combination strategies and guide future therapeutic development.

Contents

Correspondence to: Mrs. Ying Zhang, Department of Hematology, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, 314 Anshanxi Road, Nankai, Tianjin 300193, P.R. China
E-mail: lll990505@163.com

*Contributed equally

Key words: tumor microenvironment, epidermal growth factor receptor-tyrosine kinase inhibitors, non-small cell lung cancer, programmed death ligand 1, immune checkpoint inhibitors

1. Introduction

The major cellular components and mediators of the tumor microenvironment (TME), including cancer cells, immune cells [such as T cells, B cells, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs)], stromal cells [cancer-associated fibroblasts (CAFs) and tumor-associated endothelial cells], cytokines and chemokines, tumor vasculature, lymphoid tissue, as well as adipocytes and exosomes, serve a critical role in cancer initiation, progression, spread and metastasis (1-3). Interactions between the tumor stroma, especially those mediated by CAFs and other stromal components, actively promote immune evasion and malignant growth in a variety of solid tumors, including lung and breast cancer as well as other epithelial malignancies (2). Tumor-infiltrating lymphocytes are a crucial part of antitumor immunity among the immune cells found in tumors and a recent study has demonstrated that immunotherapies can alter TIL activity as well as the interactions between immune and stromal cells in the TME. The TME usually contains signals that inhibit the immune response, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells, which prevent the infiltration and killing function of immune cells (3). Breaking through this immunosuppression has long been the key to immunotherapy.

In non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) mutations, the TME manifests as an immunosuppressive phenotype. Compared with EGFR wild-type (wt) cancer, these tumors usually have a much lower tumor mutational burden, which results in a limited development of neoantigens that can successfully trigger immune recognition. Insufficient neoantigen presentation

weakens T-cell priming and activation, resulting in a relatively 'immune-cold' environment. The blunting of antitumor immune activation under such conditions is primarily responsible for the lower therapeutic benefit observed with immune checkpoint inhibitors (ICIs) in this population (4,5). However, the TME in NSCLC can be affected by chemotherapy, radiotherapy or EGFR-tyrosine kinase inhibitors (TKIs). Short-term TKI treatment excels in tumor clearance and immunological upregulation, improving the overall survival and quality of life of patients (6). Nevertheless, the immunosuppressive TME gradually emerges with time, and EGFR-TKI resistance is unavoidable (7-9). This process reveals the dynamic evolution in tumor immune microenvironment induced by EGFR-TKI treatment (10).

2. Mechanisms of resistance to EGFR-TKIs

In Asian populations, over half of patients with NSCLC had EGFR-activating mutations (11). For individuals with locally progressed or metastatic NSCLC, EGFR-TKIs are now the standard therapy regimen (12,13). Their principal mechanism is competitive binding to the ATP-binding site inside the EGFR kinase domain, which inhibits kinase autophosphorylation and downstream signaling cascades. This effect inhibits tumor cell development, proliferation and metastasis (14). With improvements in medical research, numerous EGFR-TKIs from the first to third generations are now clinically available, markedly improving patient survival rates. However, despite initial success, nearly all patients develop acquired resistance to EGFR-TKI therapy, with a median progression-free survival (mPFS) of ~1 year (15,16). Resistance to EGFR-TKIs can be divided into two categories: Primary and acquired (16). Of patients with EGFR mutations, ~30% demonstrate primary resistance at the start of initial treatment, indicating no objective response to TKI therapy; however, the mechanisms causing this resistance remain unknown. However, acquired resistance refers to disease progression that occurs following an initial response to treatment. Its causes are complicated and diverse, consisting mostly of EGFR-dependent resistance, non-EGFR-dependent resistance (induced by activation of EGFR bypass or downstream signaling pathways) and histological or phenotypic alteration (17).

EGFR-dependent drug resistance. The T790M mutation accounts for 50-60% of acquired resistance in patients treated with first- and second-generation EGFR-TKIs (18). This mutation replaces a bulky methionine (M) with a threonine (T) at position 790 in exon 20 of the EGFR gene. This substitution creates steric hindrance between the aniline moiety of EGFR-TKIs and the drug-binding site within the ATP pocket of EGFR, thereby weakening drug-binding affinity. Additional mechanisms include a marked increase in ATP binding affinity for EGFR T790M, alterations in the catalytic domain and changes in overall conformational dynamics, collectively contributing to acquired resistance to TKIs (18,19).

With the widespread usage of the third-generation EGFR-TKI osimertinib, the C797S mutation has emerged as the predominant mode of resistance. The EGFR C797S mutation, located at position 797 in exon 20 of the EGFR gene, is a missense mutation in which serine replaces cysteine. It accounts for 10-26% of second-line osimertinib resistance cases and 7% of first-line resistance cases (20). This mutation damages the ATP-binding pocket, preventing third-generation TKIs from making covalent connections with the ATP-binding domain and so losing their inhibitory function (21). The C797S mutation commonly coexists with EGFR T790M in two structural forms: Cis (EGFR T790M and C797S occur on the same allele) and trans (EGFR T790M and C797S occur on different alleles). In ~85% of instances, the EGFR C797S/T790M mutation is in the cis configuration, with ~10% of patients having the C797S/T790M trans configuration. Whether the C797S and T790M mutations form a cis structure has important biological implications since it influences the therapeutic efficacy of subsequent TKIs (17,22). In addition to the most prevalent C797S mutation, C797G is another missense mutation at the same location that causes drug resistance by affecting the binding of the drug to the residue.

Aside from the classic T790M and C797S mutations, uncommon mutations at other places within the EGFR kinase domain can also cause resistance to osimertinib, mostly through interference with drug-kinase binding. Mutations at the L718 site (such as L718Q and L718V) and the adjacent G719A mutation primarily interfere spatially, preventing the critical alanine ring in the osimertinib molecule from forming a stable bond with its binding pocket. L792 mutations (most commonly and notably L792H) directly disrupt the tight binding of osimertinib to the kinase domain. Mutations at the G796 site sterically clash with the solvent-front aromatic ring of osimertinib, preventing kinase domain binding. The G724S mutation is located in the ATP-binding region and may interfere with osimertinib binding to its target by a variety of mechanisms, including generating protein structural changes, increasing ATP affinity or maintaining the kinase activation state, resulting in drug resistance. Another form of resistance is target upregulation caused by EGFR gene amplification (17,20,23).

EGFR-independent drug resistance. Activation of bypasses and downstream pathways, such as HER2, HER3, MET, KRAS, NRAS proto-oncogene, GTPase (NRAS), BRAF, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA), AXL receptor tyrosine kinase (AXL) and insulin-like growth factor-1 receptor (IGF-1R), were often classified as 'bypass' mechanisms of resistance. These pathways enable tumor cells to activate alternative routes that engage key EGFR effectors essential for tumor cell growth and survival. The most common bypass mechanism of resistance to first- and second-generation EGFR TKIs is HER2 amplification (24,25). Downstream signaling pathways activated following receptor phosphorylation included the mitogen-activated protein kinase pathway, phosphatidylinositol 3-kinase (PI3K)/Akt pathway, phospholipase Cγ1, protein kinase C and various transcriptional regulators that modulate gene expression (26).

The MET gene encoded a receptor tyrosine kinase known as c-Met, or hepatocyte growth factor receptor, which supports cancer cell growth and survival by activating the HER3-PI3K/AKT and RAS/RAF/MEK/ERK signaling pathways, thereby bypassing the inhibitory effects of EGFR receptor signaling (18,27). The Ras-Raf-MEK-ERK pathway is crucial

for regulating cell cycle progression and proliferation. KRAS and NRAS, both members of the Ras family, could activate MEK and ERK through this signaling pathway, contributing to drug resistance (28,29). BRAF mutations, although rare in EGFR-mutant (mt) NSCLC, involve kinases located downstream of RAS in the Ras-Raf-MEK-ERK pathway and may contribute to the activation of the GFR/RAS/RAF signaling pathway (30). PIK3CA, a catalytic subunit of the PI3K family of lipid kinases, serves an oncogenic role in lung adenocarcinoma by activating the PI3K/AKT/mTOR pathway. This signaling pathway regulates key cellular functions, including growth, proliferation, metabolism, angiogenesis and metastasis, and is frequently mutated or hyperactivated in various cancers. Patients with PIK3CA mutations generally exhibit a worse prognosis and shorter median survival compared with those without these mutations (31,32). IGF-1R and AXL serve key roles in regulating cell growth, differentiation, apoptosis, transformation and other critical physiological processes through the downstream PI3K/AKT signaling pathway, thereby contributing to the development of secondary drug resistance (33,34).

Histology and phenotypic conversion. Another mechanism of drug resistance involves histological transformation within the tumor itself. Specifically, some EGFR-mt lung adenocarcinomas develop into small cell lung cancer (SCLC), accounting for 3-14% of acquired resistance cases. This transformation is confirmed by histopathological biopsy, and transformed tumors typically respond to standard SCLC treatment regimens (35-38). Research indicates that the co-deletion of Rb1 and TP53 genes constitutes the key molecular basis driving such transformation (16,39).

Tumor cells can also undergo epithelial-mesenchymal transition (EMT) and develop treatment resistance. During this process, epithelial markers are downregulated and mesenchymal markers are upregulated, resulting in increased proliferation, invasion, migration and metastatic potential. However, the exact processes underpinning EMT are unknown; this process may be driven by AXL through activation of the PI3K/AKT signaling pathway (40,41) (Fig. 1).

3. Tumor-infiltrating immune cells

$CD4^+$/$CD8^+$ T cells/Treg cells. Infiltration of $CD8^+$ T cells is lower in EGFR-mt lung adenocarcinoma (LA) compared with in EGFR-wt LA, and T cell proliferation is inhibited (42,43). It has been shown that after TKI treatment, immune cell infiltration is increased and the antitumor response is enhanced in EGFR-mt patient samples (44).

However, changes in the TME by TKI treatment appear to be dynamic. TKI treatment induces the recruitment of $CD8^+$ and $CD4^+$ T cells (45). With short-term TKI treatment, the expression levels of CD8 and granzyme B (GB) in EGFR-TKI-treated T cells co-cultured with tumor cells initially increases and then decreases with the duration of treatment, and T cells infiltration shifts from enhanced to suppressed (10). Evidence has shown that following TKI resistance, the tumor can transform into a 'hot' tumor with increased immune cell infiltration, with notable effector and T cell infiltration (46).

In the early stages of treatment, sensitive EGFR-TKIs can increase the number of cytotoxic $CD8^+$ T cells, while short-term EGFR inhibition reduces the proportion of $Foxp3^+$ Tregs. However, these changes in the TME, which favor immune-mediated combination therapies for cancer, may gradually diminish with continued treatment (47).

TAMs. TAMs serve an important role in tumor progression in EGFR-mt NSCLC, and their enrichment in human NSCLC is associated with poor clinical outcomes (48). How M1 and M2 type macrophages are distinguished may be influenced by the TME and macrophages may exhibit a mixed phenotype of both types of markers.

EGFR mutations promote the expansion of alveolar macrophages (AM), but TKI treatment markedly reduces AM numbers in tumor-bearing mice (48). In EGFR-mt cells, M2 macrophages increase, and while short-term TKI treatment reduces M2 infiltration, the number of M2 macrophages rise again with the onset of TKI resistance (10,45).

DCs. EGFR-mt lung cancer (LC) drives the immune phenotype of tumor-infiltrating DCs (TIDC) toward an immunosuppressive direction and is closely associated with exosomes. Tumor-derived exosomes (TEXs) bridge the interaction between tumor cells and immune cells, thereby altering antitumor immune responses. Research has shown that TEXs can inhibit myeloid differentiation, promoting the transformation of monocytes from immune-stimulating to immune-suppressive cells, thereby supporting immune evasion (49). There is also evidence that EGFR activation by TEXs weakens the innate immunity of the host (50) and promotes tumor metastasis (51).

Compared with EGFR-wt tumor-bearing mice, TIDCs and lymph node (LN) DCs isolated from EGFR-19del tumor-bearing mice produced markedly less IL-12p40. This finding suggests that EGFR-19del Lewis LC tumors drive the process of immunosuppression by affecting DCs in both the tumor and LN. Furthermore, EGFR-mt LC cells may influence DC function by secreting exosomes *in vitro*. These exosomes not only accelerate tumor growth but also induce immune suppression (43). However, short-term TKI treatment markedly increases DC infiltration within the TME (47).

Natural killer (NK) cells. In EGFR-mt tumors, both the innate and adaptive lymphocyte compartments exhibit signs of functional exhaustion. The proportion of cytotoxic NK cells is reduced, whereas NK T cell (NKT) subsets with low cytotoxic potential are markedly increased (52,53). These NKT subsets display diminished expression of activation and cytotoxicity-related genes, indicating weakened innate immune cytotoxicity within the EGFR-mt TME.

In EGFR-mt LC, TKI therapy increases NK cell infiltration and cytotoxicity (44,54). Elevated IL-6 levels are linked to decreased immune cell infiltration during short-term TKI treatment. On the other hand, NK cell activity is decreased in EGFR-mt NSCLC with acquired TKI resistance due to increased upregulation of IL-6 (55).

B cells. Tertiary lymphoid structures (TLS) are primarily composed of B lymphocytes, which are essential to their development (53). TLS are associated with a higher response

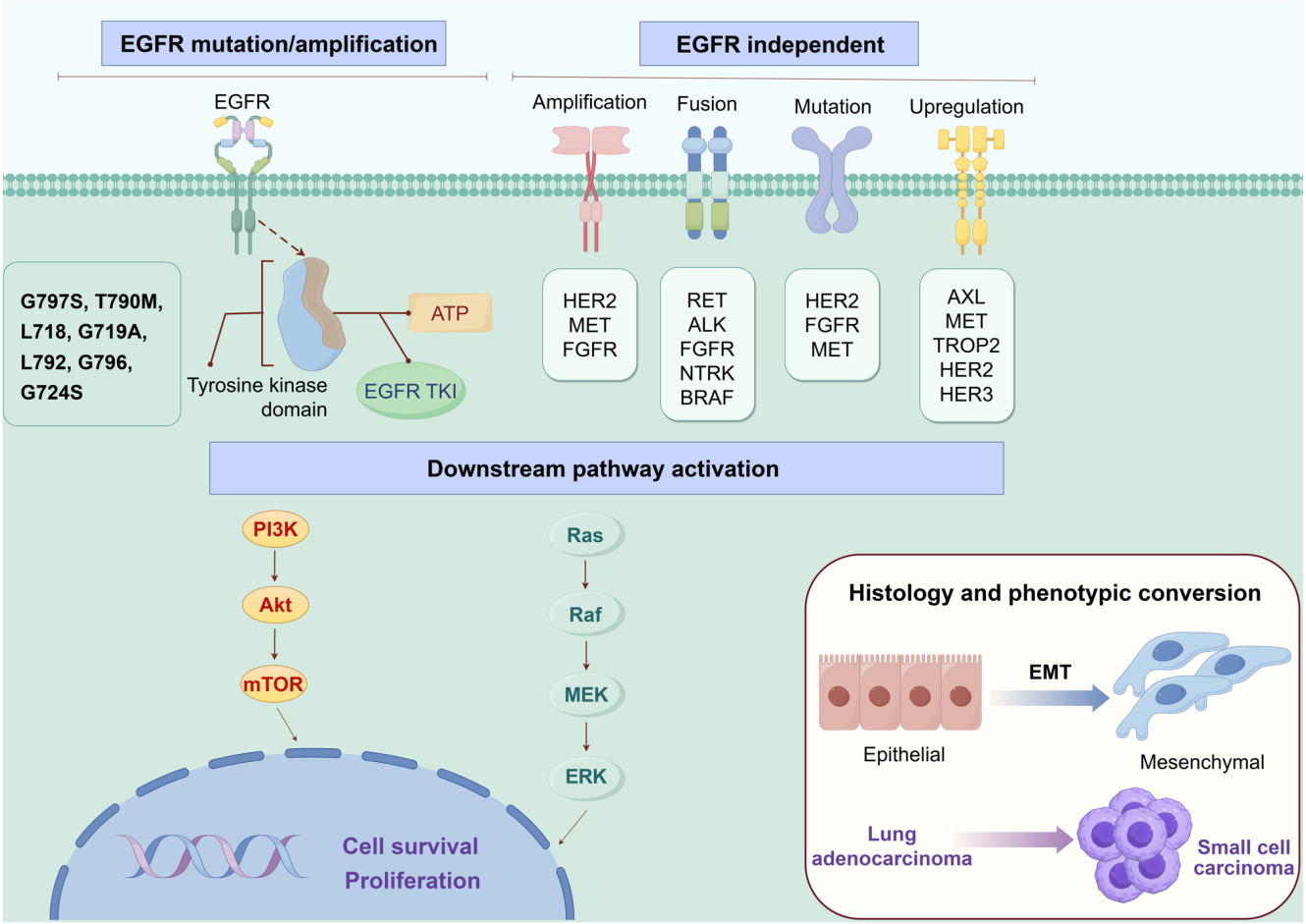

Figure 1. The illustration outlines the key mechanisms of resistance to EGFR-TKIs in EGFR-mutant NSCLC. EGFR-dependent resistance arises from evolution of the target itself (e.g., T790M/C797S mutations or gene amplification), which restores EGFR kinase activity and re-activates the downstream PI3K/Akt/mTOR and Ras/Raf/MEK/ERK pathways. EGFR independent resistance occurs via bypass activation or downstream nodal mutations, circumventing EGFR inhibition and ultimately converging on the same core pathways. In addition, tumors may evade therapy through histology or phenotypic transformation, such as transformation to SCLC or EMT. EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; EMT, epithelial-mesenchymal transition.

to immunotherapies and increased survival. In the TME of EGFR-negative LUAD, B cells build the TLS (56). Tumor-infiltrating B cells and antigen presentation-related markers were notably lower in EGFR-mt tumors compared with in EGFR-wt tumors, according to single-cell transcriptome analysis, which resulted in decreased T-cell activation (52). Immune infiltration, including B cells and $CD8^+$ T cells, is increased in TKI-responsive samples but not in resistant ones after EGFR-TKI treatment, indicating that combining ICIs may be more advantageous prior to the development of TKI resistance (44).

4. Immunomodulatory molecules

Programmed death ligand 1 (PD-L1). PD-L1 is a critical immune checkpoint protein that is broadly expressed on the surface of tumor cells and tumor-infiltrating immune cells. It inhibits anticancer immune responses by binding to programmed cell death protein-1 (PD-1) on T cells, B cells, DCs and NK T cells, and is an indicator of poor survival in most advanced cancers (57-59). It is regulated in NSCLC by two distinct mechanisms: Driver genetic alterations and inflammation. Previous research has reported the dynamic relationship between EGFR mutation status and PD-L1 expression before and after EGFR-TKI, but the exact relationship remains controversial. Meanwhile, it is worth noting that there may be an association between the levels of PD-L1 expression and the efficacy of EGFR-TKI therapy (60).

Compared with EGFR-wt, EGFR-mt typically results in upregulation of PD-L1 (59,61-65), a process that is closely associated with activation of the IL-6/JAK/STAT3 and p-ERK1/2/p-c-Jun signaling pathways (66,67). However, one clinical study found that EGFR-mt patients exhibit relatively low PD-L1 expression (16%; PD-L1 ≥5%) before TKI treatment (68). An analysis involving 18 studies and 3,969 patients revealed that, compared with EGFR-wt tumors, EGFR-mt NSCLC is less likely to display PD-L1 positivity (65).

EGFR-TKI not only directly inhibits the activity of tumor cells, but also indirectly enhances the antitumor immune response by downregulating PD-L1 (61,66). After short-term TKI treatment, PD-L1 expression is markedly reduced (10), which can be monitored by immuno-positron emission tomography imaging (69-71). This downregulation of PD-L1 may be partly dependent on the activation of the NF-kB pathway

or AKT-STAT3 pathway (62,63). However, a previous study suggested the opposite conclusion, proposing that TKI treatment increases PD-L1 expression in patients with EGFR-mt NSCLC (72). As reported in one study (73), PD-L1 expression showed marked changes following EGFR-TKI treatment, with the proportion of patients with PD-L1 strong positive tumors [tumor proportion score (TPS) ≥50%] increasing from 14% at baseline to 28% after TKI treatment. With subsequent ICI treatment, patients with high PD-L1 expression (TPS ≥50%) achieved a longer mPFS compared with those with low PD-L1 expression [TPS <50%; 7.1 vs. 1.7 months; HR=0.18 (0.04-0.56); P=0.0033]. The PD-1 signaling pathway is activated in EGFR-TKI resistant tumors (74). After long-term TKI treatment, the expression levels of PD-1 typically increase (10,72). As EGFR-TKI resistance develops, the proportion of patients with PD-L1 strong positive tumors increases from baseline (73). A retrospective analysis showed that PD-L1 expression levels changed during the development of resistance in 16 patients (28%), of which 12 patients had higher PD-L1 expression levels after resistance (68). In addition, changes in PD-L1 expression levels in tumor-infiltrating immune cells between baseline and the development of EGFR-TKI resistance were also of interest, with the proportion of patients with ≥10% PD-L1 expression in tumor-infiltrating immune cells increasing from 11% at baseline to 25% after TKI treatment.

Evidence suggests that patients with EGFR-TKI resistance, particularly those who are T790M negative, may derive greater benefit from PD-1 inhibitors, in part due to their higher PD-L1 expression levels (75). This suggests that further research is needed to determine whether patients with EGFR-TKI resistance can benefit from PD-1 inhibitor therapy. Due to the potential efficacy of immunotherapy in patients with active PD-1 pathways, investigating the combined effects of immunotherapy and its interactions with TKIs after TKI resistance may help to optimize treatment strategies for these patients.

MHC-II, CD40, CD80 and CD86. LN DC and AM in EGFR-mt tumor-bearing mice exhibit an immunosuppressive phenotype compared with EGFR-wt tumor-bearing mice, characterized by downregulation of MHC-II and the costimulatory molecules CD40, CD80, and CD86 (43,48).

CD47. Integrin-associated protein (CD47) is a cell surface immunoglobulin-like molecule that inhibits phagocytosis by interacting with signal regulatory protein α on phagocytes. CD47 is selectively upregulated in patients with EGFR mutations. After treatment with TKI, CD47 expression is downregulated, which promotes DC phagocytosis of NSCLC cells. However, after the development of resistance *in vitro*, CD47 expression is upregulated (76).

Cytokines and chemokines. Cytokines and chemokines in the TME are also regulated by EGFR-TKIs. According to molecular research, cytokines can modulate immunological signaling by causing target-cell receptors to undergo structural or functional changes, such as receptor breakage or shedding (77-79). In patients with EGFR-mt NSCLC, the pro-inflammatory cytokine IL-17A was highly expressed, along with markedly increased expression of transforming growth factor-β (TGF-β), which are closely associated with cell proliferation and induction of TKI resistance (80,81). Compared with AM in control mice, AM in hormone mice produced more cytokines (such as IL-1α and TNF-α) and chemokines [such as CXC motif chemokine ligand (CXCL) 1 and CXCL2] (48). In addition, the chemokine CXCL2 secreted by CAFs is considered to induce the expression of PD-L1 in LA cells, thereby indirectly modulating tumor immunity (82).

TKI treatment promotes the transformation of EGFR-mt NSCLC from a 'cold' to a 'hot' tumor. After EGFR-TKI treatment, the level of type I interferon (IFN) is notably increased in EGFR-mt human LC cell lines, and the expression of the chemokines CXCL9, CXCL10 and CXCL11 is also markedly upregulated. Meanwhile, the CXCL10/CXCR3 pathway is activated in the EGFR-mt LC transgenic mouse model (10,42,55). Furthermore, an analysis of serum IFN-γ levels in 20 patients with NSCLC treated with EGFR-TKI revealed that serum IFN-γ levels were markedly higher compared with baseline levels in treated patients (66).

In EGFR-TKI-resistant patients, the TME remodels from a non-inflammatory to an inflammatory state. Studies have shown that acquired resistance to TKI therapy can promote an inflammatory response, with the IFN-γ pathway becoming markedly enriched after TKI resistance, along with higher levels of granzyme A expression (46). Type I IFN levels are decreased in resistant cell lines, whereas expression of the pro-inflammatory cytokines IL-6 and TGF-β1 is notably increased (10,81,83,84).

CD24 and lymphocyte-activation gene 3 (LAG-3). In a related study (85), the expression of the innate immune checkpoint CD24 was found to be upregulated in EGFR-mt cells *in vitro* following EGFR-TKI treatment, which is consistent with the observation after TKI resistance. These findings suggest that EGFR inhibition in EGFR-mt NSCLC cells promotes the development of a TME conducive to immune escape. Furthermore, the expression of the checkpoint protein LAG-3 is markedly elevated after EGFR-TKI treatment (86).

5. Immunotherapy clinical trials in EGFR-mt advanced NSCLC

Immunological monotherapy or dual therapy. A pair of meta-analyses (87,88), which included studies such as CheckMate057, KEYNOTE-010, OAK and POPLAR, demonstrated that the efficacy of immunotherapy monotherapy was inferior compared with that of docetaxel monotherapy in patients with EGFR-mt LC. As a result, patients with EGFR-mt NSCLC are less likely to derive notable benefits from immunotherapy monotherapy. The BIRCH study (89), a phase II, single-arm, multicenter clinical trial evaluating the efficacy and safety of atezolizumab monotherapy in advanced PD-L1-expressing NSCLC, included 45 patients with EGFR mutations. The results showed that atezolizumab exhibited antitumor activity regardless of EGFR status and was particularly effective in the high PD-L1-expressing subgroup (TC3 or IC3). However, even among EGFR-mt patients with high PD-L1 expression, the objective response rate (ORR) and median overall survival (mOS) remained notably lower compared with those in EGFR-wt patients. The ATLANTIC (90) study was a

single-arm, open-label, phase II trial evaluating durvalumab (an anti-PD-L1 monoclonal antibody) as a third-line or later treatment for advanced NSCLC, enrolling a total of 77 patients with EGFR mutations. Subgroup analysis revealed that while the ORR in patients with EGFR-mt NSCLC with PD-L1 expression ≥25% was higher compared with those with low PD-L1 expression [12.2% (9/74) vs. 3.6% (1/28)], it was still lower compared with EGFR-wt patients [16.4% (24/146) vs. 7.5% (7/93)].

In one cohort of the KEYNOTE-021 study (91), a total of 11 patients were enrolled. The combination of ipilimumab [anti-cytotoxic T-lymphocyte associated protein 4 (CTLA-4) antibody] and pembrolizumab showed limited efficacy in patients with EGFR-mt NSCLC, with an ORR of only 10%, substantially lower than the 30% observed in EGFR-wt patients. Overall, the data suggest that while the combination therapy exhibits some antitumor activity in patients with advanced NSCLC who had received multiple lines of therapy, it was less effective and more toxic in EGFR-mt patients, with a 64% incidence of treatment-emergent adverse events (AEs), including 29% of grade 3-5 severe AEs. The study suggests that dual immunotherapy has limited effectiveness in EGFR-mt patients and must be chosen with caution, especially when PD-L1 expression levels are low. In conclusion, these clinical trial data indicate that the overall response rate to immunotherapy is lower in patients with EGFR-mt NSCLC compared with those with EGFR-wt. Nevertheless, antitumor activity was observed in EGFR-mt patients with high PD-L1 expression, suggesting that high PD-L1 expression may serve as an important predictor of potential benefit from immunotherapy in this subgroup.

Immunotherapy combined with targeted therapy. CAURAL (92) is a phase II clinical trial investigating the combination of osimertinib (a third-generation EGFR-TKI) and durvalumab in patients with advanced NSCLC with EGFR T790M mutations who had progressed after prior EGFR-TKI treatment. The primary objective of the trial was to evaluate safety, with efficacy assessed as an exploratory endpoint. The results demonstrated an ORR of 64% in the combination therapy arm, lower than the 80% observed in the osimertinib monotherapy arm, failing to show an advantage over single-agent therapy. Nonetheless, the trial was terminated early due to the elevated risk of interstitial lung disease observed in related studies. This highlights that safety issues need to be considered when combining an EGFR-TKI with an ICI.

Immunotherapy combined with chemotherapy. CT18 (93) is a multicenter, single-arm, phase II clinical trial evaluating the efficacy and safety of toripalimab (anti-PD-1 antibody) in combination with carboplatin and pemetrexed in patients (n=40) with advanced EGFR-mt NSCLC who had failed EGFR-TKI therapy and lacked T790M mutations. The results showed an ORR of 50.0% (95% CI, 33.8-66.2), and a disease control rate (DCR) of 87.5% (95% CI, 73.2-95.8). The mPFS was 7.0 months (95% CI, 4.8-8.4), while the mOS reached 23.5 months (95% CI, 18.0 to NR months). The overall safety profile of the treatment was manageable, with 97.5% of patients experiencing treatment-related AEs (TRAEs), of which 65.0% were grade 3 or higher, most commonly bone marrow suppression, elevated transaminases and nausea. Immune-related AEs occurred in 40% of patients, with only 5.0% being grade 3 or higher.

CheckMate722 (94) is a phase III clinical trial designed for patients with advanced NSCLC characterized by EGFR mutations and resistance to TKI therapy (n=294). This investigation sought to compare the efficacy of nivolumab combined with chemotherapy against chemotherapy alone. While the combination therapy group showed a modest improvement in mPFS (5.6 vs. 5.4 months; HR=0.75), the difference fell short of statistical significance. Likewise, the combination treatment did not notably extend mOS (19.4 vs. 15.9 months; HR=0.82). The ORR was 31.3 and 26.7% in the combination and chemotherapy groups, respectively. Post-hoc subgroup analyses revealed a potential trend of enhanced PFS in specific subpopulations, including those with sensitizing EGFR mutations or patients treated exclusively with first-line TKI therapy, with HRs of 0.72 and 0.64, respectively.

The KEYNOTE-789 study (95), a randomized, double-blind phase III trial in 492 patients with TKI-resistant, EGFR-mt advanced NSCLC, demonstrated that pembrolizumab in combination with chemotherapy only slightly improved mPFS (5.6 vs. 5.5 months; HR=0.80; P=0.0122) or mOS (15.9 vs. 14.7 months; HR=0.84; P=0.0362) compared with chemotherapy alone. Neither the CheckMate722 nor the KEYNOTE-789 trial met their primary endpoints, suggesting that merely combining anti-PD-1 ICIs with chemotherapy may not provide meaningful clinical benefits for this patient population. Consequently, it is essential to further investigate potential biomarkers and refine combination treatment strategies to enhance therapeutic outcomes.

Immunotherapy combined with chemotherapy + anti-angiogenic drugs. IMpower150 (96,97) is a phase III clinical trial that enrolled 123 patients with EGFR-mt NSCLC to assess the efficacy of three regimens of atezolizumab in combination with carboplatin and paclitaxel (ACP), bevacizumab plus carboplatin and paclitaxel (BCP) and atezolizumab plus bevacizumab plus carboplatin and paclitaxel (ABCP). The results showed that patients with EGFR mutations or ALK translocations in the ABCP group demonstrated a longer mPFS compared with those in the BCP group, at 9.7 vs. 6.1 months, respectively (HR=0.59; 95% CI, 0.37-0.94; P=0.025). The mOS for the ABCP group was 29.4 months, which was notably superior to the 18.1 months observed in the BCP group (HR=0.60; 95% CI, 0.31-1.14), while the ACP group (19.0 months) was similar to the BCP group (HR=1.00; 95% CI, 0.57-1.74). In terms of safety, 100% of patients in the ABCP group experienced TRAEs, of which 66.7% were grade 3/4, although no grade 5 events were reported. The incidence of TRAE in the ACP and BCP groups was 88.6% (with 56.8% grade 3/4) and 95.3% (55.8% grade 3/4), respectively. In summary, the IMpower150 study demonstrated that the ABCP regimen substantially prolonged OS in patients with EGFR-mt NSCLC with controllable AEs and had a favorable safety and tolerability profile.

ORIENT-31 (98) is a phase III clinical trial designed to assess the efficacy of sintilimab ± IBI305 combined with chemotherapy (pemetrexed + cisplatin) in patients with

locally advanced or metastatic EGFR-mt non-squamous NSCLC who have progressed after EGFR-TKI treatment. The randomized trial with 476 patients indicated that sintilimab combined with chemotherapy significantly improved mPFS, with 5.5 vs. 4.3 months for chemotherapy alone (HR=0.72; 95% CI, 0.55-0.94; P=0.016). Sintilimab plus IBI305 and chemotherapy extended mPFS to 7.2 months, compared with 4.3 months for chemotherapy alone (HR=0.51; 95% CI, 0.39-0.67; P<0.0001), demonstrating significant benefit. Regarding mOS, the sintilimab plus IBI305 combination chemotherapy group had a mOS of 21.1 months (95% CI, 17.5-23.9), which was similar to the 19.2 months observed in the chemotherapy alone group (HR=0.98; 95% CI, 0.72-1.34; P=0.8883), with no statistically significant difference. The mOS for the sintilimab plus chemotherapy group was 20.5 months (95% CI, 15.8-25.3), which was comparable to the chemotherapy alone group at 19.2 months (HR=0.97; 95% CI, 0.71-1.32; P=0.8202). In terms of safety, 56% of patients (88/158) in the sintilimab plus IBI305 plus chemotherapy group developed a grade 3 or higher TRAE, which was notably higher compared with in the sintilimab plus chemotherapy (41%) and chemotherapy alone (49%) groups. Despite immunotherapy side effects (such as immune pneumonitis and rash) were more common, overall safety and tolerability were satisfactory and most AEs could be mitigated with appropriate management.

IMpower151 (99) is a phase III study that included 305 patients with advanced non-squamous NSCLC. The trial assessed the efficacy of atezolizumab in combination with bevacizumab (an anti-VEGF monoclonal antibody) and chemotherapy (carboplatin + pemetrexed) as a first-line treatment. Preliminary findings indicated that the combination therapy (ABCP) showed limited benefits in mPFS and mOS compared with the control group (BCP), without reaching statistical significance. The mPFS was 9.5 and 7.1 months (HR=0.84; 95% CI, 0.65-1.09; P=0.18) and the mOS was 20.7 and 18.7 months (HR=0.93; 95% CI, 0.67-1.28) for the two groups, respectively. These results suggest that although the combination of immunotherapy and chemotherapy provided a slight survival benefit for patients with EGFR-mt NSCLC, the effect was modest compared with other NSCLC subtypes and did not substantially alter the prognosis. Safety analyses revealed a high incidence of AEs in both groups and no new safety signals. The rates of all-cause AEs were 99.3% in the ABCP group (with 66.4% being grade 3/4) and 100% in the BCP group (61.4% grade 3/4).

ATTLAS (100) is a phase III study performed in Korea that enrolled 225 patients with stage IV NSCLC diagnosed with EGFR sensitizing mutations or ALK translocations, including the ABCP group (n=151) and the PC group (n=74). All patients had disease progression or intolerance to one or more EGFR or ALK TKIs. A total of 168 patients with EGFR-mt NSCLC who were resistant to EGFR-TKI were enrolled in the study, 109 in the ABCP arm and 59 in the PC arm. The results demonstrated that the ABPC arm significantly improved PFS compared with the PC arm, with mPFS of 8.48 vs. 5.62 months, respectively [HR=0.62 (95% CI, 0.45-0.86); P=0.004]. Subgroup analysis of patients with EGFR-TKI-resistant EGFR-mt NSCLC revealed similar findings, with mPFS of 8.7 months in the ABCP arm and 5.6 months in the PC arm (HR=0.60; 95% CI, 0.43-0.84; P=0.002). However, no notable OS benefit was observed in either group. In terms of safety, the incidence of grade 3 or higher TRAEs was higher in the ABCP arm compared with in the PC arm, primarily related to cytotoxic chemotherapy. Nevertheless, bevacizumab-related TRAEs were generally manageable with appropriate supportive care.

HARMONi-A (101,102) is a phase III clinical trial performed in China, enrolling 322 patients with advanced or metastatic EGFR-mt NSCLC who experienced disease progression during EGFR-TKI treatment. This study evaluated the efficacy of ivonescimab in combination with chemotherapy compared with chemotherapy alone. In the trial, eligible patients were randomized 1:1 to receive ivonescimab plus chemotherapy (pemetrexed and carboplatin) or placebo plus chemotherapy. Results showed that ivonescimab plus chemotherapy resulted in a significant improvement in mPFS compared with the chemotherapy group at 7.1 and 4.8 months, respectively [HR=0.46 (95% CI; 0.34-0.62); P<0.001]. The ORRs of the two groups were 50.6 and 35.4%, respectively, and the DCRs were 93.1 and 83.2%, respectively. In terms of safety, the incidence of AEs was 99.4 and 97.5% in the two groups, and the incidence of grade 3 or higher treatment-emergent AEs was 61.5 and 49.1%, respectively.

In addition, two ongoing phases II clinical trials (103,104) have shown preliminary efficacy. A single-arm phase II study enrolled 64 patients with EGFR-mt NSCLC who had progressed following EGFR-TKI treatment and received combination chemotherapy with PM8002/BNT327, a bispecific antibody targeting PD-L1 and VEGF-A. The results revealed an overall ORR of 54.7% (95% CI, 41.8-67.2). In patients with a TPS ≥50%, the ORR reached 92.3% (95% CI, 64.0-99.8), indicating that the antitumor activity of PM8002/BNT327 treatment was positively associated with tumor PD-L1 expression levels. Additionally, cohort 5 (EGFR-TKI-resistant cohort) of the DUBHE-L-201 study (104) included 31 patients. The cohort received QL1706 + carboplatin + pemetrexed + bevacizumab administered intravenously on day 1 of a 21-day cycle for four cycles. Maintenance therapy was QL1706 + pemetrexed + bevacizumab. QL1706 is a bifunctional MabPair product containing anti-PD-1 and anti-CTLA-4 antibodies. The primary endpoint of the study was safety and secondary endpoints include confirmed ORR, investigator-assessed duration of remission (DoR), PFS and OS. Preliminary results showed a median DoR of 11.3 months (95% CI, 4.2-19.9), PFS of 8.5 months (95% CI, 5.7-13.3) and OS of 26.5 months (95% CI, 12.8-not evaluable) (Table I).

6. Discussion and future perspectives

In summary, TKI-treated and TKI-resistant TMEs show a shift towards a 'hot' tumor with increased immune cell infiltration (Fig. 2), with an increase in the immune-activating components of the TME, including increased numbers or upregulation of tumor-infiltrating immune cells, immunomodulatory molecules, cytokines or chemokines and a reduction or impairment of immunosuppressive components. Notably, acquired EGFR-TKI resistance promotes immune escape in lung cancer by upregulating PD-L1 expression. Detailed studies have shown that changes in the TME following TKI

Table I. ICI-based immunotherapy combinations for EGFR-mutant advanced NSCLC.

NCT	Clinical trial	Phase	Intervention	Result	(Refs.)
A, Immunotherapy combined with targeted therapy					
NCT02454933	CAURAL	III	Osi + Durva (n=14) Osi (n=15)	ORR:64% vs. 80%	(92)
B, Immunotherapy combined with chemotherapy					
NCT03513666	CT18	II	Tori + Carbo + Pem (n=40)	ORR=50.0% (95% CI, 33.8-66.2) DCR=87.5% (95%CI, 73.2-95.8) mPFS=7.0 m (95% CI, 4.8-8.4) mOS=23.5 m (95% CI, 18.0-NR)	(93)
NCT02864251	CheckMate722	III	Nivo + Chemo (n=144) Chemo (n=150)	mPFS:5.6 m vs. 5.4 m (HR=0.75; 95% CI, 0.56-1.00) mOS:19.4 m vs. 15.9 m (HR=0.82; 95% CI, 0.61-1.10)	(94)
NCT03515837	KEYNOTE-789	III	Pembro + Chemo (n=245) Placebo + Chemo (n=247)	mPFS:5.6 mvs5.5 m (HR=0.80; 95% CI, 0.65-0.97) mOS:15.9 vs. 14.7 (HR=0.84; 95% CI, 0.69-1.02)	(95)
C, Immunotherapy combined with chemotherapy + anti-angiogenic drugs					
NCT02366143	IMpower150	III	ABCP: Atezo + Bev + Carbo+ Pac (n=34) BCP: (n=44) ACP: (n=45)	mPFS: ABCP vs. BCP:9.70 m vs. 6.1 m (HR=0.59; 95% CI, 0.37-0.94) mOS: ABCP vs. BCP:26.1 m vs. 20.3 m (HR=0.91; 95% CI, 0.53-1.59) ACP vs. BCP: 21.4 m vs. 20.3 m (HR=1.16; 95% CI, 0.71-1.89)	(96,97)
NCT03802240	ORIENT-31	III	SIC: Sinti + IBI305 + Chemo (n=158) SC: Sinti + Chemo (n=158) C: Chemov (n=160)	mPFS: SIC vs. C:7.2 m vs. 4.3 m (HR=0.51; 95% CI, 0.39-0.67) SC vs. C: 5.5 m vs. 4.3 m (HR=0.72; 95% CI, 0.55-0.94) mOS: SIC: 21.1 m (95% CI) 17.5-23.9) SC:20.5 m (95% CI) 15.8-25.3) C:19.2 m (95% CI (15.8-22.4)	(98)
NCT04194203	IMpower151	III	ABCP: (n=152) BCP: (n=153)	mPFS: 9.5 m vs. 7.1 m (HR=0.84; 95% CI, 0.65-1.09; P=0.18) mOS: 20.7 m vs. 18.7 m (HR=0.93; 95% CI, 0.67-1.28)	(99)
NCT03991403	ATTLAS	III	ABPC: Atezo + Bev + Pac + Carbo (n=109) PC: (n=59)	mPFS: ABCP vs. PC:8.71 m vs. 5.62 m (HR=0.60; 95% CI, 0.43-0.84)	(100)
NCT05184712	HARMONi-A	III	ivonescimab + Chemo (n=161) Chemo (n=161)	mPFS: 7.1 m vs. 4.8 m (HR=0.46; 95% CI, 0.34-0.62)	(101, 102)
NCT05756972	-	II	PM8002/BNT327 + Carbo + Pem (n=64)	ORR:54.7%	(103)

Table I. Continued.

C, Immunotherapy combined with chemotherapy + anti-angiogenic drugs					
NCT	Clinical trial	Phase	Intervention	Result	(Refs.)
NCT05329025	DUBHE-L-201	II	QL1706+ Carbo + Pem + Bev (n=31)	mDoR, PFS, and OS were 11.3 (95% CI, 4.2-19.9), 8.5 (5.7-13.3), and 26.5 months (12.8-not evaluable), respectively	(104)

ICs, immune cells; EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; NSCLC, non-small cell lung cancer; mPFS, median progression-free survival; mOS, median overall Survival; RR, response rate; ORR, objective response rate; TPS, tumor proportion score; TTP, time to progression; DCR, disease control rate; TCs, tumor cells; mDoR, median duration of remission; ICI, immune checkpoint inhibitor; NCT, ClinicalTrials.gov identifier; Osi, Osimertinib; Durva, Durvalumab; Tori, Toripalimab; Carbo, Carboplatin; Pem, Pemetrexed; Nivo, Nivolumab; Chemo, Chemotherapy; Pembro, Pembrolizumab; Atezo, Atezolizumab; Bev, Bevacizumab; Pac, Paclitaxel; Sinti, Sintilimab.

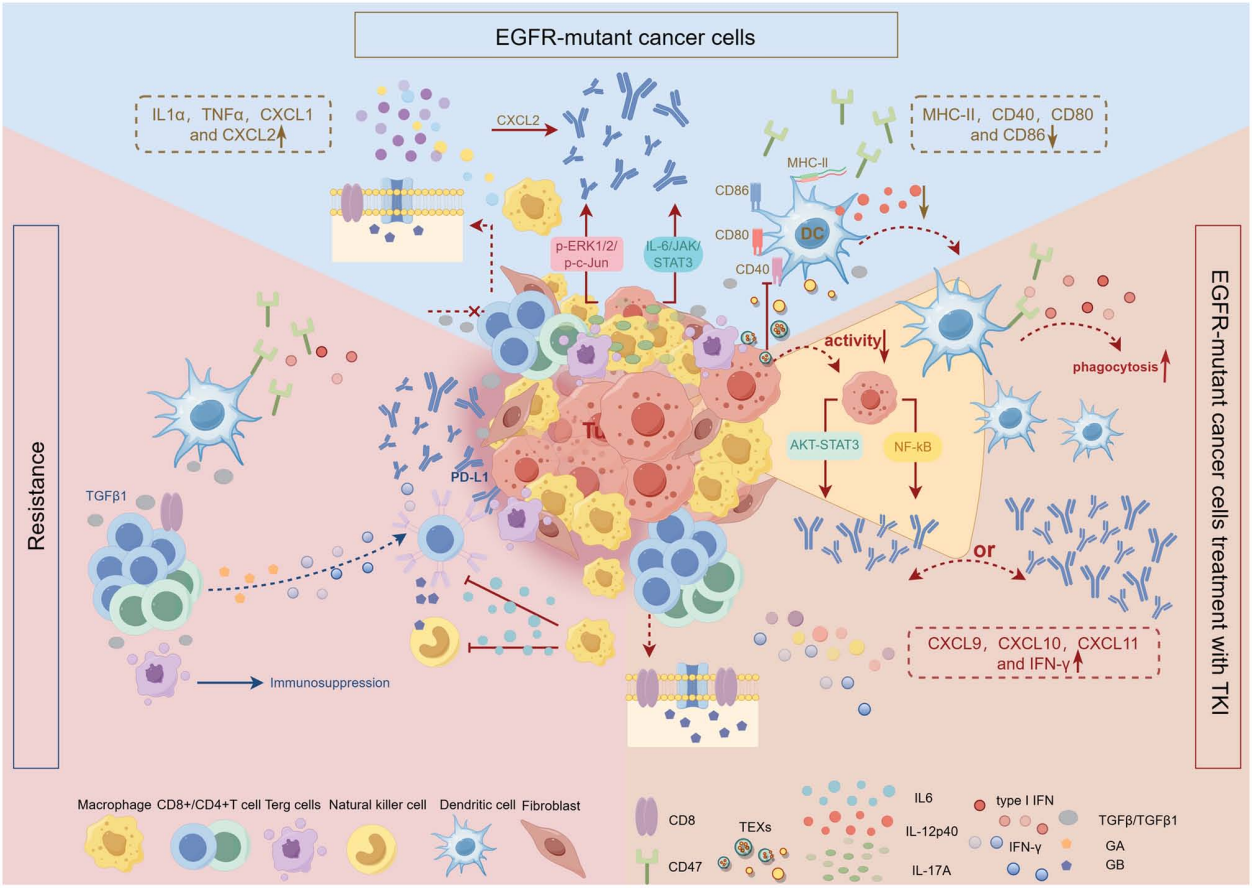

Figure 2. TME in EGFR-mutant NSCLC and its dynamics during short-term TKI treatment and resistance. The TME of EGFR-mutant NSCLC is immunosuppressive. Low $CD8^+$ T-cell infiltration and inhibition of their proliferation and activation by the cytokine TGF-β; EGFR mutation promotes AM proliferation; TEXs inhibit DC function and reduce IL-12p40 production; MHC-II and co-stimulatory molecules of DCs and AMs (CD40, CD80, CD86) expression were downregulated; CD47 was selectively overexpressed; EGFR mutations upregulated PD-L1 via IL-6/JAK/STAT3 and p-ERK1/2/p-c-Jun pathways; high expression of pro-inflammatory cytokine IL-17A; increased expression of cytokines TGF-β, IL1α and TNFα and chemokines CXCL1 and CXCL2; and CAFs release of CXCL2 induced PD-L1 expression. EGFR-TKI can directly inhibit the activity of tumor cells. Short-term TKI treatment transformed 'cold tumors' into 'hot tumors', with an increase in T-cell and DC infiltration, an increase in CD8 and GB expression levels Compared with pre-treatment and a marked decrease in TAM infiltration and $Foxp3^+$ Tregs; CD47 is downregulated, enhancing DC-mediated phagocytosis; PD-L1 expression could be reduced or upregulated, and the reduced PL-L1 was considered to be associated with the NF-κB and AKT-STAT3 pathways; the level of type I IFNs (IFN-α, IFN-β) was increased and the expression of IFN-γ and the chemokines CXCL9, CXCL10, and CXCL11 was upregulated. Upon TKI resistance, the TME was remodeled towards inflammation, with enrichment of inflammatory and IFN-γ pathway; CD8 and GB expression decreased and granzyme A expression increased; TAM numbers rise; CD47 was re-upregulated; the PD-1 pathway was activated and the expression level of PD-L1 was increased; type I IFN decreased and the expression of IL-6 and TGF-β1 was increased; the increased expression of IL-6 suppressed T cell and NK cell cytotoxicity and GB marker expression. TME, tumor microenvironment; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; TGF-β, transforming growth factor-β; AM, alveolar macrophages; CXCL, CXC motif chemokine ligand; TEXs, tumor-derived exosomes; p-, phosphorylated; IL, interleukin; TNF, tumor necrosis factor; CAFs, cancer-associated fibroblasts; GB, granzyme B; IFN, interferon; Tregs, regulatory T cells; DC, dendritic cell; TAMs, tumor-associated macrophages; NK, natural killer; PD-L1, programmed death ligand 1; TKI, tyrosine kinase inhibitor.

Table II. Association between PD-L1 expression and treatment efficacy of EGFR-TKI in EGFR-mutant NSCLC patients.

First author/s,year	Gen.	EGFR-TKI	Country	EGFR-mutant (PD-L1 Testing) Patients (n)	PD-L1 expression in EGFR-mutant/after TKIs	Associated clinical outcomes	(Refs.)
D'Incecco *et al*, 2015	I	Gefitinib or erlotinib	Italian	95/123	PD-L1 positivity (2+ or 3+) in >5% of TCs: 55.3%/-	PD-L1+: higher RR (61.2% vs. 34.8%, P=0.001), longer TTP (11.7 m vs. 5.7 m, P<0.0001), and longer OS (21.9m vs. 12.5 m, P=0.09)	(64)
Lin *et al*, 2015		Gefitinib or erlotinib	China	56	Positive in 53.6% of tumor specimens/-	PD-L1+: higher DCR (93.3% vs. 61.5%, P=0.004), longer mPFS (16.5 vs. 8.6 m, P=0.001) and mOS (35.3 vs. 19.8 m, P=0.004)	(106)
Soo *et al*, 2017	I/II	Erlotinib, gefitinib, or dacomitinib	South Korea	90	≥1% positive: ICs 44%, TCs 59%/no association	PD-L1+: shorter PFS [HR=1.008 (1.001-1.015), P=0.017]	(107)
Yoneshima *et al*, 2018		-	Japan	71	TPS of ≥1%: 42.3%/-	TPS ≥1% vs. <1%: shorter mPFS (9 vs. 14 m, P=0.016)	(108)
Su *et al*, 2018		-	China	101	Positive (TC3/IC3, TC1-2/ IC1-2): 35.6%/-	TC3/IC3 vs. TC1-2/IC1-2 vs. TC0/IC0: lower ORR (35.7% vs. 63.2% vs. 67.3%, P=0.002), shorter mPFS (3.8 vs. 6.0 vs. 9.5m, P<0.001), and higher primary resistance rates (66.7% vs. 30.2%) P=0.009)	(109)
Hsu *et al*, 2019		Gefitinib, erlotinib, or afatinib	China	123	TPS of ≥1%: 30.1%/-	TPS ≥1% vs. <1%: shorter mPFS (2.1 vs. 7.3 m, P<0.001) and mOS (11.2 vs. 38.2, P=0.002), higher primary resistance rates (OR=5.95, 95% CI 2.35-15.05, P<0.001)	(110)
Yang *et al*, 2020		Gefitinib, erlotinib, or afatinib	China	153	TPS 1-49%: 25.5%, TPS ≥50%: 11.8%/Stable in 60% (9/15), increased in 40% upon progression	TPS 0% vs. 1-49% vs. ≥50%: ORR (65.6% vs. 56.4% vs. 38.9%, P<0.001), DCR (93.8% vs. 97.4% vs. 55.6%, P<0.001), mPFS (2.5 vs. 12.8 vs. 5.9 m, P=0.027), primary resistance rates (6.3% vs. 2.6% vs. 44.4%, P<0.001)	(60)

Table II. Contiued.

First author/s,year	Gen.	EGFR-TKI	Country	EGFR-mutant (PD-L1 Testing) Patients (n)	PD-L1 expression in EGFR-mutant/after TKIs	Associated clinical outcomes	(Refs.)
Isomoto *et al*, 2020	I/II/III	Gefitinib, erlotinib, afatinib, dacomitinib or osimertinib	Japan	134	TPS of ≥50%: 14%/TPS of ≥50%: 28%	-	(73)
Brown *et al*, 2018	I/III	Osimertinib vs. gefitinib/ erlotinib	Global	128	TC ≥1%: 51%/-	TC ≥1% vs. <1%: Osimertinib group (n=54): similar mPFS (18.4 vs. 18.9 m); gefitinib/erlotinib group (n=52): shorter mPFS (6.9 vs. 10.9 m)	(105)
Sakata *et al*, 2021	III	Osimertinib	Japan	538	TPS ≥50%: 11.9%, 1-49%: 31.6%, <1%: 30%, Unknown: 26.6%/-	mPFS: TPS ≥50%: 11.1 m (95% CI 8.3-NR) [HR=2.24 (1.17-4.30), P=0.015], TPS 1-49%:14.7 m (95% CI 13.6-20.5) [HR=1.66 (1.05-2.63), P=0.029], TPS <1%: NR (95% CI 20.7-NR)	(111)
Alves *et al*, 2022	I/III	Gefitinib, erlotinib or osimertinib	Brazil	278/188	TC ≥1%: 36.7%/-	Higher PD-L1 (TPS ≥50% vs. 1-49% vs. <1%): not associated with mOS (37.5 vs. 46.5 vs. 50.4 m, P=0.48), and decreased event-free survival (median:9 vs. 34 vs. 26 m, P=0.014)	(112)
Papazyan *et al*, 2024	III	Osimertinib	France	96	TPS ≥50%: 20.8%/ decreased in 4/15 patients at relapse	TPS ≥50% vs. <50%: shorter mPFS (9.3 vs. 17.5 m, P=0.044), and mOS (14.3 vs. 26.0 m, P=0.025), ORR (53.3% vs. 46.5%, P>0.9)	(113)
Lakkuna-rajah *et al*, 2023		Osimertinib	Canada	231	TPS ≥50%: 29.4%/-	TPS ≥50% vs. <1%: shorter PFS (HR=1.59, 95% CI 1.07-2.36, P=0.023), and OS (HR=1.82, 95% CI 1.10-2.99, P=0.019)	(114)
Yoshimura *et al*, 2021		Osimertinib	Japan	71	TPS ≥50%: 21.1%/-	TPS ≥50% vs. <50%: lower ORR (53.3% vs. 81.1%, P=0.043) and DCR (73.3% vs. 98.1%, P=0.007), shorter mPFS (5.0 vs. 17.4 m, P<0.001); TPS ≥50% vs. 1-49% vs. <1%: higher primary resistance rates (33.33% vs. 3.85% vs3.45%, P=0.006)	(115)

Table II. Continued.

First author/s,year	Gen.	EGFR-TKI	Country	EGFR-mutant (PD-L1 Testing) Patients (n)	PD-L1 expression in EGFR-mutant/after TKIs	Associated clinical outcomes	(Refs.)
Hsu *et al*, 2022		Osimertinib	China	85/71	TPS ≥50%: 9.4%/-	TPS ≥50% vs. <50%: shorter mPFS [9.7 vs. 26.5 m, aHR=0.19 (0.06-0.67), P=0.009], shorter mOS [25.4 vs. NR, aHR=0.09 (0.01-0.70), P=0.021]	(116)
Hamakawa *et al*, 2023		Osimertinib	Japan	64	TPS ≥20%: 34.4%/-	TPS ≥20% vs. <20%: shorter mPFS (9.1 vs. 28.1 m, log-rank P=0.013), with PD-L1 TPS ≥20% associated with early resistance to osimertinib	(117)

PD-L1, programmed death-ligand; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer; PFS, progression-free survival; OS, overall survival; RR, response rate; ORR, objective response rate; TPS, tumor proportion score; TTP, time to progression; DCR, disease control rate; TCs, tumor cells; ICs, immune cells; TC3/IC3: ≥50% for TC or ≥10% for IC, TC1-2/IC1-2: 5-49% for TC or 5-9% for IC, TC0/IC0: <5% for TC or IC.

treatment are dynamic, with short-term effects suggesting a potential therapeutic window during which TKI treatment could more effectively leverage the immune system to target tumors (Fig. 3). However, the long-term effects of TKI therapy are more complex and may be influenced by various factors, such as treatment cycles, drug sensitivity and the specific treatment regimen employed.

Previous reports have shown that patients with high PD-L1 expression have a greater survival benefit following treatment with first-generation EGFR-TKI. However, the subsequent FLAURA trial (105) reported conflicting results, suggesting a shorter PFS in patients with high PD-L1 expression. With second and third-generation TKI therapies, high PD-L1 expression has been associated with worse survival and higher rates of drug resistance. These findings suggest a strong relationship between PD-L1 expression levels and TKI efficacy. Previous cases are described in Table II (106-117). However, discrepancies between studies may be due to differences in drug generations, PD-L1 detection methods and thresholds for defining PD-L1 expression levels. Most existing studies are retrospective, with limited prospective studies and notable regional variability. There is an urgent need for larger, multicenter, prospective clinical trials to validate the relationship between PD-L1 expression and clinical outcomes. Standardization of PD-L1 detection methods is also critical to identifying the optimal population for ICI treatments, ultimately providing greater clinical benefit to patients.

Recent research has focused on patients with high PD-L1 expression. Available evidence suggests that immunotherapy can offer notable benefits to patients, both during short-term TKI therapy and after the development of resistance. However, the effect of short-course TKI therapy on PD-L1 expression levels has long been a topic of debate. The timing of immunotherapy interventions and the monitoring of their efficacy need to be further confirmed. On the one hand, the immunological characteristics of the TME, such as inflammatory or immunosuppressive status, as well as the level of PD-L1 expression should guide the choice of treatment. On the other hand, identifying reliable biomarkers, such as PD-L1 and cytokine levels, is crucial for accurately predicting treatment response. It is particularly critical and urgent to conduct large-scale, multicenter clinical trials to validate the role of these biomarkers and to promote their standardized application in clinical practice.

The growing importance of immunotherapy is further underscored by the emergence of resistance to TKI therapy. In patients with high PD-L1 expression and EGFR-TKI resistance, immunotherapy may provide a notable survival benefit (73,103). Conversely, in patients with low PD-L1 expression and EGFR-TKI resistance, immunotherapy interventions do not yield the desired therapeutic outcomes. Fourth-generation EGFR-TKI and antibody-drug conjugates (ADCs) offer novel therapeutic strategies for patients who have failed conventional TKI therapy and exhibit low PD-L1 expression. Fourth-generation TKIs are designed to overcome the limitations of third-generation TKIs in the face of drug resistance. ADCs targeting trophoblast surface antigen 2 have shown notable therapeutic efficacy in immunotherapy non-responders. Future studies should focus on elucidating the underlying mechanisms of dynamic immune changes in the TME, as well as performing comprehensive and systematic

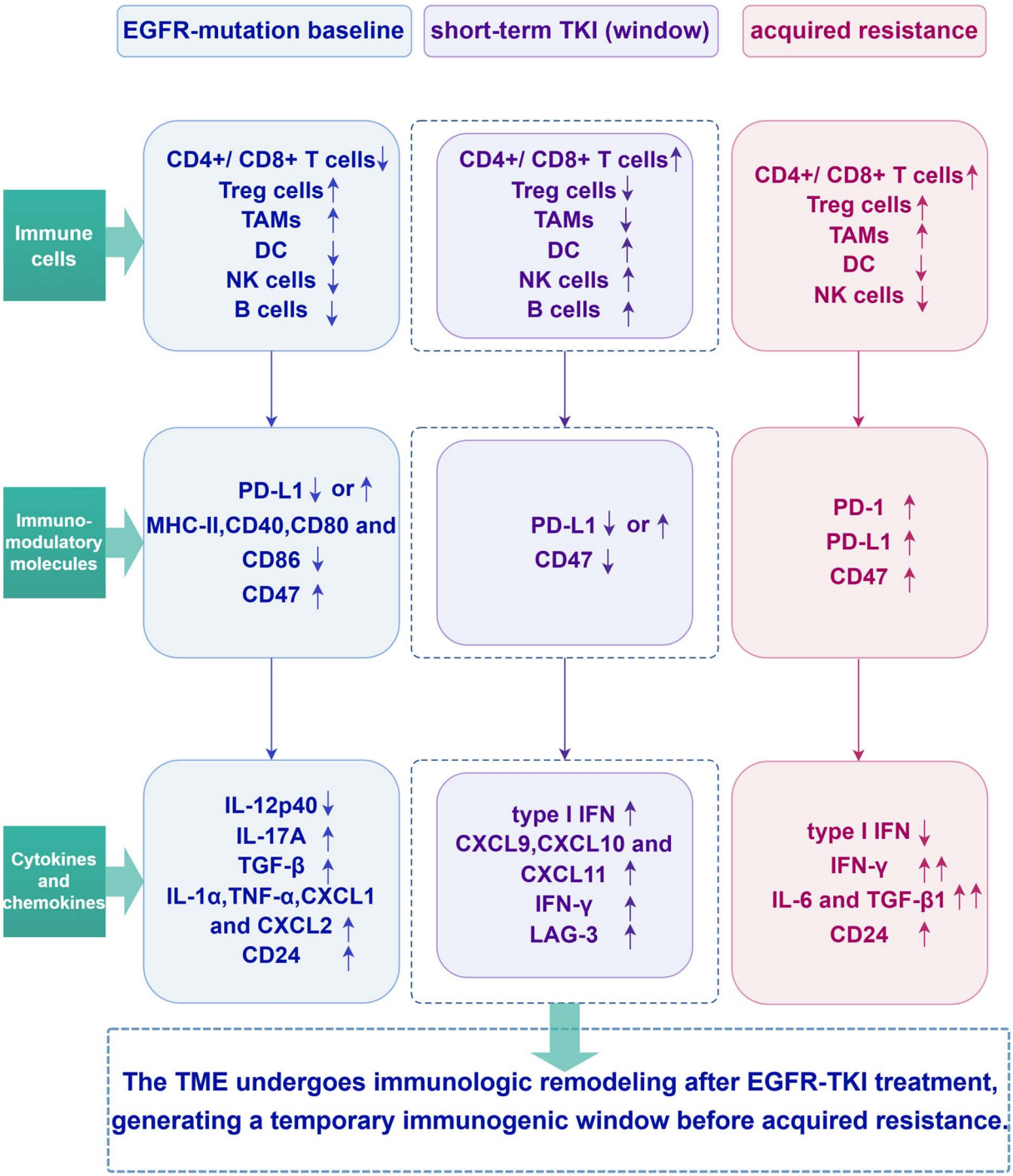

Figure 3. A timeline schematic illustrating dynamic changes in the TME from the EGFR-mutant baseline state through short-term TKI exposure to the development of acquired resistance. TME, tumor microenvironment; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; Treg cells, regulatory T cells; TAMs, tumor-associated macrophages; DC, dendritic cell; NK, natural killer; PD-L1, programmed death ligand 1; IL, interleukin; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; CXCL, CXC motif chemokine ligand; IFN, interferon; LAG-3, lymphocyte-activation gene 3; PD-1, programmed cell death protein-1.

evaluations of combination therapies that include TKIs, ICIs, ADCs and anti-angiogenic drugs. Moreover, actively exploring the optimal therapeutic window and addressing the safety issues associated with combination therapies will greatly advance the field of oncology and bring new hope to more patients.

Acknowledgements

No applicable.

Funding

The present study was supported by the Renxin Medical Research Program of the Beijing Weiai Public Welfare Foundation (grant no. RXYS2025-0200630118), the Scientific Research Program of the Tianjin Municipal Education Commission (grant no. 2025ZD061), and the Scientific Research Program of the Hebei Administration of Traditional Chinese Medicine (grant no. T2026091).

Availability of data and materials

Not applicable.

Authors' contributions

All authors contributed to the study conception and design. HM and LL conceived the study and wrote and revised the manuscript. CJ, YC and JH wrote the manuscript. HM, LL, QT and CJ wrote, reviewed and edited the manuscript. DY contributed to the manuscript preparation. YZ supervised the study. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Osipov A, Saung MT, Zheng L and Murphy AG: Small molecule immunomodulation: The tumor microenvironment and overcoming immune escape. J Immunother Cancer 7: 224, 2019.
2. Vuletic A, Mirjacic Martinovic K and Jurisic V: The role of tumor microenvironment in triple-negative breast cancer and its therapeutic targeting. Cells 14: 1353, 2025.
3. Kraja FP, Jurisic VB, Hromić-Jahjefendić A, Rossopoulou N, Katsila T, Mirjacic Martinovic K, De Las Rivas J, Diaconu CC and Szöőr Á: Tumor-infiltrating lymphocytes in cancer immunotherapy: From chemotactic recruitment to translational modeling. Front Immunol 16: 1601773, 2025.
4. Vryza P, Fischer T, Mistakidi E and Zaravinos A: Tumor mutation burden in the prognosis and response of lung cancer patients to immune-checkpoint inhibition therapies. Transl Oncol 38: 101788, 2023.
5. To KKW, Fong W and Cho WCS: Immunotherapy in treating EGFR-mutant lung cancer: Current challenges and new strategies. Front Oncol 11: 635007, 2021.
6. Jurisic V, Vukovic V, Obradovic J, Gulyaeva LF, Kushlinskii NE and Djordjević N: EGFR polymorphism and survival of NSCLC patients treated with TKIs: A systematic review and meta-analysis. J Oncol 2020: 1973241, 2020.
7. Li K, Quan L, Huang F, Li Y and Shen Z: ADAM12 promotes the resistance of lung adenocarcinoma cells to EGFR-TKI and regulates the immune microenvironment by activating PI3K/Akt/mTOR and RAS signaling pathways. Int Immunopharmacol 122: 110580, 2023.
8. Jeong HO, Lee H, Kim H, Jang J, Kim S, Hwang T, Choi DW, Kim HS, Lee N, Lee YM, *et al*: Cellular plasticity and immune microenvironment of malignant pleural effusion are associated with EGFR-TKI resistance in non-small-cell lung carcinoma. iScience 25: 105358, 2022.
9. Liu L, Wang C, Li S, Bai H and Wang J: Tumor immune microenvironment in epidermal growth factor receptor-mutated non-small cell lung cancer before and after epidermal growth factor receptor tyrosine kinase inhibitor treatment: A narrative review. Transl Lung Cancer Res 10: 3823-3839, 2021.
10. Lu C, Gao Z, Wu D, Zheng J, Hu C, Huang D, He C, Liu Y, Lin C, Peng T, *et al*: Understanding the dynamics of TKI-induced changes in the tumor immune microenvironment for improved therapeutic effect. J Immunother Cancer 12: e009165, 2024.
11. Corvaja C, Passaro A, Attili I, Aliaga PT, Spitaleri G, Signore ED and De Marinis F: Advancements in fourth-generation EGFR TKIs in EGFR-mutant NSCLC: Bridging biological insights and therapeutic development. Cancer Treat Rev 130: 102824, 2024.
12. Owen DH, Ismaila N, Ahluwalia A, Feldman J, Gadgeel S, Mullane M, Naidoo J, Presley CJ, Reuss JE, Singhi EK and Patel JD: Therapy for stage IV non-small cell lung cancer with driver alterations: ASCO living guideline, version 2024.3. J Clin Oncol 43: e2-e16, 2025.
13. Obradović J, Niševic-Lazović J, Sekeruš V, Milašin J, Perin B and Jurisic V: Investigating the frequencies of EGFR mutations and EGFR single nucleotide polymorphisms genotypes and their predictive role in NSCLC patients in Republic of Serbia. Mol Biol Rep 52: 350, 2025.
14. Yamaoka T, Ohba M and Ohmori T: Molecular-targeted therapies for epidermal growth factor receptor and its resistance mechanisms. Int J Mol Sci 18: 2420, 2017.
15. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, Kris MG, Miller VA, Ladanyi M and Riely GJ: Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res 19: 2240-2247, 2013.
16. Wu SG and Shih JY: Management of acquired resistance to EGFR TKI-targeted therapy in advanced non-small cell lung cancer. Mol Cancer 17: 38, 2018.
17. Zalaquett Z, Catherine Rita Hachem M, Kassis Y, Hachem S, Eid R, Raphael Kourie H and Planchard D: Acquired resistance mechanisms to osimertinib: The constant battle. Cancer Treat Rev 116: 102557, 2023.
18. Westover D, Zugazagoitia J, Cho BC, Lovly CM and Paz-Ares L: Mechanisms of acquired resistance to first- and second-generation EGFR tyrosine kinase inhibitors. Ann Oncol 29 (Suppl 1): i10-i19, 2018.
19. Ko B, Paucar D and Halmos B: EGFR T790M: Revealing the secrets of a gatekeeper. Lung Cancer (Auckl) 8: 147-159, 2017.
20. Leonetti A, Sharma S, Minari R, Perego P, Giovannetti E and Tiseo M: Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. Br J Cancer 121: 725-737, 2019.
21. Thress KS, Paweletz CP, Felip E, Cho BC, Stetson D, Dougherty B, Lai Z, Markovets A, Vivancos A, Kuang Y, *et al*: Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. Nat Med 21: 560-562, 2015.
22. Niederst MJ, Hu H, Mulvey HE, Lockerman EL, Garcia AR, Piotrowska Z, Sequist LV and Engelman JA: The allelic context of the C797S mutation acquired upon treatment with third-generation EGFR inhibitors impacts sensitivity to subsequent treatment strategies. Clin Cancer Res 21: 3924-3933, 2015.
23. Passaro A, Jänne PA, Mok T and Peters S: Overcoming therapy resistance in EGFR-mutant lung cancer. Nat Cancer 2: 377-391, 2021.
24. Proto C, Lo Russo G, Corrao G, Ganzinelli M, Facchinetti F, Minari R, Tiseo M and Garassino MC: Treatment in EGFR-mutated non-small cell lung cancer: How to block the receptor and overcome resistance mechanisms. Tumori 103: 325-337, 2017.
25. Du X, Yang B, An Q, Assaraf YG, Cao X and Xia J: Acquired resistance to third-generation EGFR-TKIs and emerging next-generation EGFR inhibitors. Innovation (Camb) 2: 100103, 2021.
26. Roy V and Perez EA: Beyond trastuzumab: Small molecule tyrosine kinase inhibitors in HER-2-positive breast cancer. Oncologist 14: 1061-1069, 2009.
27. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, *et al*: MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 316: 1039-1043, 2007.
28. Punekar SR, Velcheti V, Neel BG and Wong KK: The current state of the art and future trends in RAS-targeted cancer therapies. Nat Rev Clin Oncol 19: 637-655, 2022.
29. Ohashi K, Sequist LV, Arcila ME, Lovly CM, Chen X, Rudin CM, Moran T, Camidge DR, Vnencak-Jones CL, Berry L, *et al*: Characteristics of lung cancers harboring NRAS mutations. Clin Cancer Res 19: 2584-2591, 2013.
30. Simanshu DK, Nissley DV and McCormick F: RAS proteins and their regulators in human disease. Cell 170: 17-33, 2017.
31. Polivka J and Janku F: Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. Pharmacol Ther 142: 164-175, 2014.

32. Eng J, Woo KM, Sima CS, Plodkowski A, Hellmann MD, Chaft JE, Kris MG, Arcila ME, Ladanyi M and Drilon A: Impact of concurrent PIK3CA mutations on response to EGFR tyrosine kinase inhibition in EGFR-mutant lung cancers and on prognosis in oncogene-driven lung adenocarcinomas. J Thorac Oncol 10: 1713-1719, 2015.
33. Pollak M: Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 8: 915-928, 2008.
34. Namba K, Shien K, Takahashi Y, Torigoe H, Sato H, Yoshioka T, Takeda T, Kurihara E, Ogoshi Y, Yamamoto H, *et al*: Activation of AXL as a preclinical acquired resistance mechanism against osimertinib treatment in EGFR-mutant non-small Cell Lung Cancer Cells. Mol Cancer Res 17: 499-507, 2019.
35. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, Bergethon K, Shaw AT, Gettinger S, Cosper AK, *et al*: Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 3: 75ra26, 2011.
36. Oser MG, Niederst MJ, Sequist LV and Engelman JA: Transformation from non-small-cell lung cancer to small-cell lung cancer: Molecular drivers and cells of origin. Lancet Oncol 16: e165-e172, 2015.
37. Yin X, Li Y, Wang H, Jia T, Wang E, Luo Y, Wei Y, Qin Z and Ma X: Small cell lung cancer transformation: From pathogenesis to treatment. Semin Cancer Biol 86: 595-606, 2022.
38. Li Y, Xie T, Wang S, Yang L, Hao X, Wang Y, Hu X, Wang L, Li J, Ying J and Xing P: Mechanism exploration and model construction for small cell transformation in EGFR-mutant lung adenocarcinomas. Signal Transduct Target Ther 9: 261, 2024.
39. Lee JK, Lee J, Kim S, Kim S, Youk J, Park S, An Y, Keam B, Kim DW, Heo DS, *et al*: Clonal history and genetic predictors of transformation into small-cell carcinomas from lung adenocarcinomas. J Clin Oncol 35: 3065-3074, 2017.
40. Huang L and Fu L: Mechanisms of resistance to EGFR tyrosine kinase inhibitors. Acta Pharm Sin B 5: 390-401, 2015.
41. Neel DS and Bivona TG: Secrets of drug resistance in NSCLC exposed by new molecular definition of EMT. Clin Cancer Res 19: 3-5, 2013.
42. Sumimoto H, Takano A, Igarashi T, Hanaoka J, Teramoto K and Daigo Y: Oncogenic epidermal growth factor receptor signal-induced histone deacetylation suppresses chemokine gene expression in human lung adenocarcinoma. Sci Rep 13: 5087, 2023.
43. Yu S, Sha H, Qin X, Chen Y, Li X, Shi M and Feng J: EGFR E746-A750 deletion in lung cancer represses antitumor immunity through the exosome-mediated inhibition of dendritic cells. Oncogene 39: 2643-2657, 2020.
44. Fang Y, Wang Y, Zeng D, Zhi S, Shu T, Huang N, Zheng S, Wu J, Liu Y, Huang G, *et al*: Comprehensive analyses reveal TKI-induced remodeling of the tumor immune microenvironment in EGFR/ALK-positive non-small-cell lung cancer. Oncoimmunology 10: 1951019, 2021.
45. Lin Z, Wang Q, Jiang T, Wang W and Zhao JJ: Targeting tumor-associated macrophages with STING agonism improves the antitumor efficacy of osimertinib in a mouse model of EGFR-mutant lung cancer. Front Immunol 14: 1077203, 2023.
46. Chen Q, Xia L, Wang J, Zhu S, Wang J, Li X, Yu Y, Li Z, Wang Y, Zhu G and Lu S: EGFR-mutant NSCLC may remodel TME from non-inflamed to inflamed through acquiring resistance to EGFR-TKI treatment. Lung Cancer 192: 107815, 2024.
47. Jia Y, Li X, Jiang T, Zhao S, Zhao C, Zhang L, Liu X, Shi J, Qiao M, Luo J, *et al*: EGFR-targeted therapy alters the tumor microenvironment in EGFR-driven lung tumors: Implications for combination therapies. Int J Cancer 145: 1432-1444, 2019.
48. Wang DH, Lee HS, Yoon D, Berry G, Wheeler TM, Sugarbaker DJ, Kheradmand F, Engleman E and Burt BM: Progression of EGFR-mutant lung adenocarcinoma is driven by alveolar macrophages. Clin Cancer Res 23: 778-788, 2017.
49. Valenti R, Huber V, Iero M, Filipazzi P, Parmiani G and Rivoltini L: Tumor-released microvesicles as vehicles of immunosuppression. Cancer Res 67: 2912-2915, 2007.
50. Gao L, Wang L, Dai T, Jin K, Zhang Z, Wang S, Xie F, Fang P, Yang B, Huang H, *et al*: Tumor-derived exosomes antagonize innate antiviral immunity. Nat Immunol 19: 233-245, 2018.
51. Zhang H, Deng T, Liu R, Bai M, Zhou L, Wang X, Li S, Wang X, Yang H, Li J, *et al*: Exosome-delivered EGFR regulates liver microenvironment to promote gastric cancer liver metastasis. Nat Commun 8: 15016, 2017.
52. Cho JW, Park S, Kim G, Han H, Shim HS, Shin S, Bae YS, Park SY, Ha SJ, Lee I and Kim HR: Dysregulation of T_{FH}-B-T_{RM} lymphocyte cooperation is associated with unfavorable anti-PD-1 responses in EGFR-mutant lung cancer. Nat Commun 12: 6068, 2021.
53. Zhao J, Lu Y, Wang Z, Wang H, Zhang D, Cai J, Zhang B, Zhang J, Huang M, Pircher A, *et al*: Tumor immune microenvironment analysis of non-small cell lung cancer development through multiplex immunofluorescence. Transl Lung Cancer Res 13: 2395-2410, 2024.
54. He S, Yin T, Li D, Gao X, Wan Y, Ma X, Ye T, Guo F, Sun J, Lin Z and Wang Y: Enhanced interaction between natural killer cells and lung cancer cells: Involvement in gefitinib-mediated immunoregulation. J Transl Med 11: 186, 2013.
55. Patel SA, Nilsson MB, Yang Y, Le X, Tran HT, Elamin YY, Yu X, Zhang F, Poteete A, Ren X, *et al*: IL6 Mediates suppression of T- and NK-cell function in EMT-associated TKI-resistant EGFR-mutant NSCLC. Clin Cancer Res 29: 1292-1304, 2023.
56. Yang L, He YT, Dong S, Wei XW, Chen ZH, Zhang B, Chen WD, Yang XR, Wang F, Shang XM, *et al*: Single-cell transcriptome analysis revealed a suppressive tumor immune microenvironment in EGFR mutant lung adenocarcinoma. J Immunother Cancer 10: e003534, 2022.
57. Chen J, Jiang CC, Jin L and Zhang XD: Regulation of PD-L1: A novel role of pro-survival signalling in cancer. Ann Oncol 27: 409-416, 2016.
58. Zhang Y, Wang L, Li Y, Pan Y, Wang R, Hu H, Li H, Luo X, Ye T, Sun Y and Chen H: Protein expression of programmed death 1 ligand 1 and ligand 2 independently predict poor prognosis in surgically resected lung adenocarcinoma. Onco Targets Ther 7: 567-573, 2014.
59. Azuma K, Ota K, Kawahara A, Hattori S, Iwama E, Harada T, Matsumoto K, Takayama K, Takamori S, Kage M, *et al*: Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. Ann Oncol 25: 1935-1940, 2014.
60. Yang CY, Liao WY, Ho CC, Chen KY, Tsai TH, Hsu CL, Su KY, Chang YL, Wu CT, Hsu CC, *et al*: Association between programmed death-ligand 1 expression, immune microenvironments, and clinical outcomes in epidermal growth factor receptor mutant lung adenocarcinoma patients treated with tyrosine kinase inhibitors. Eur J Cancer 124: 110-122, 2020.
61. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, Mikse OR, Cherniack AD, Beauchamp EM, Pugh TJ, *et al*: Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov 3: 1355-1363, 2013.
62. Lin K, Cheng J, Yang T, Li Y and Zhu B: EGFR-TKI down-regulates PD-L1 in EGFR mutant NSCLC through inhibiting NF-κB. Biochem Biophys Res Commun 463: 95-101, 2015.
63. Abdelhamed S, Ogura K, Yokoyama S, Saiki I and Hayakawa Y: AKT-STAT3 pathway as a downstream target of EGFR signaling to regulate PD-L1 expression on NSCLC cells. J Cancer 7: 1579-1586, 2016.
64. D'Incecco A, Andreozzi M, Ludovini V, Rossi E, Capodanno A, Landi L, Tibaldi C, Minuti G, Salvini J, Coppi E, *et al*: PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. Br J Cancer 112: 95-102, 2015.
65. Soo RA, Lim SM, Syn NL, Teng R, Soong R, Mok TSK and Cho BC: Immune checkpoint inhibitors in epidermal growth factor receptor mutant non-small cell lung cancer: Current controversies and future directions. Lung Cancer 115: 12-20, 2018.
66. Chen N, Fang W, Zhan J, Hong S, Tang Y, Kang S, Zhang Y, He X, Zhou T, Qin T, *et al*: Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-driven NSCLC: Implication for optional immune targeted therapy for NSCLC patients with EGFR mutation. J Thorac Oncol 10: 910-923, 2015.
67. Zhang N, Zeng Y, Du W, Zhu J, Shen D, Liu Z and Huang JA: The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. Int J Oncol 49: 1360-1368, 2016.
68. Gainor JF, Shaw AT, Sequist LV, Fu X, Azzoli CG, Piotrowska Z, Huynh TG, Zhao L, Fulton L, Schultz KR, *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 22: 4585-4593, 2016.
69. Li D, Zou S, Cheng S, Song S, Wang P and Zhu X: Monitoring the response of PD-L1 expression to epidermal growth factor receptor tyrosine kinase inhibitors in nonsmall-cell lung cancer xenografts by immuno-PET imaging. Mol Pharm 16: 3469-3476, 2019.
70. He H, Qi X, Fu H, Xu J, Zheng Q, Chen L, Zhang Y, Hua H, Xu W, Xu Z, *et al*: Imaging diagnosis and efficacy monitoring by [^{89}Zr] Zr-DFO-KN035 immunoPET in patients with PD-L1-positive solid malignancies. Theranostics 14: 392-405, 2024.

71. Huang W, Zhou J, Liu Y, Yang Y, Saladin RJ, Hsu JC, Cai W and Kang L: Advances in immunoPET/SPECT imaging: The role of Fab and $F(ab')_2$ fragments in theranostics. Acta Pharm Sin B 15: 3888-3924, 2025.
72. Xing S, Hu K and Wang Y: Tumor immune microenvironment and immunotherapy in non-small cell lung cancer: Update and new challenges. Aging Dis 13: 1615-1632, 2022.
73. Isomoto K, Haratani K, Hayashi H, Shimizu S, Tomida S, Niwa T, Yokoyama T, Fukuda Y, Chiba Y, Kato R, *et al*: Impact of EGFR-TKI treatment on the tumor immune microenvironment in EGFR mutation-positive non-small cell lung cancer. Clin Cancer Res 26: 2037-2046, 2020.
74. Wang S, Rong R, Yang DM, Fujimoto J, Bishop JA, Yan S, Cai L, Behrens C, Berry LD, Wilhelm C, *et al*: Features of tumor-microenvironment images predict targeted therapy survival benefit in patients with EGFR-mutant lung cancer. J Clin Invest 133: e160330, 2023.
75. Haratani K, Hayashi H, Tanaka T, Kaneda H, Togashi Y, Sakai K, Hayashi K, Tomida S, Chiba Y, Yonesaka K, *et al*: Tumor immune microenvironment and nivolumab efficacy in EGFR mutation-positive non-small-cell lung cancer based on T790M status after disease progression during EGFR-TKI treatment. Ann Oncol 28: 1532-1539, 2017.
76. Nigro A, Ricciardi L, Salvato I, Sabbatino F, Vitale M, Crescenzi MA, Montico B, Triggiani M, Pepe S, Stellato C, *et al*: Enhanced expression of CD47 is associated with off-target resistance to tyrosine kinase inhibitor gefitinib in NSCLC. Front Immunol 10: 3135, 2020.
77. Jurisic V: Multiomic analysis of cytokines in immuno-oncology. Expert Rev Proteomics 17: 663-674, 2020.
78. Jurisić V, Bogdanovic G, Srdic T, Jakimov D, Mrdjanovic J, Baltic M and Baltic VV: Modulation of TNF-alpha activity in tumor PC cells using anti-CD45 and anti-CD95 monoclonal antibodies. Cancer Lett 214: 55-61, 2004.
79. Jurisic V, Srdic-Rajic T, Konjevic G, Bogdanovic G and Colic M: TNF-α induced apoptosis is accompanied with rapid CD30 and slower CD45 shedding from K-562 cells. J Membr Biol 239: 115-122, 2011.
80. Lee KL, Lai TC, Lee WJ, Chen YC, Ho KH, Hung WY, Yang YC, Chan MH, Hsieh FK, Chung CL, *et al*: Sustaining the activation of EGFR signal by inflammatory cytokine IL17A prompts cell proliferation and EGFR-TKI resistance in lung cancer. Cancers (Basel) 15: 3288, 2023.
81. Huang H, Zhu X, Yu Y, Li Z, Yang Y, Xia L and Lu S: EGFR mutations induce the suppression of $CD8^+$ T cell and anti-PD-1 resistance via ERK1/2-p90RSK-TGF-β axis in non-small cell lung cancer. J Transl Med 22: 653, 2024.
82. Hong SH, Kang N, Kim O, Hong SA, Park J, Kim J, Lee MA and Kang J: EGFR-tyrosine kinase inhibitors induced activation of the autocrine CXCL10/CXCR3 pathway through crosstalk between the tumor and the microenvironment in EGFR-mutant lung cancer. Cancers (Basel) 15: 124, 2022.
83. Mirjačić Martinović K, Vuletić A, Tišma Miletić N, Besu Žižak I, Milovanović J, Matković S and Jurišić V: Circulating cytokine dynamics as potential biomarker of response to anti-PD-1 immunotherapy in BRAFwt MM patients. Transl Oncol 38: 101799, 2023.
84. Inoue C, Miki Y, Saito R, Hata S, Abe J, Sato I, Okada Y and Sasano H: PD-L1 induction by cancer-associated fibroblast-derived factors in lung adenocarcinoma cells. Cancers (Basel) 11: 1257, 2019.
85. Shiiya A, Noguchi T, Tomaru U, Ariga S, Takashima Y, Ohhara Y, Taguchi J, Takeuchi S, Shimizu Y, Kinoshita I, *et al*: EGFR inhibition in EGFR-mutant lung cancer cells perturbs innate immune signaling pathways in the tumor microenvironment. Cancer Sci 114: 1270-1283, 2023.
86. Zhou J, Yu X, Hou L, Zhao J, Zhou F, Chu X, Wu Y, Zhou C and Su C: Epidermal growth factor receptor tyrosine kinase inhibitor remodels tumor microenvironment by upregulating LAG-3 in advanced non-small-cell lung cancer. Lung Cancer 153: 143-149, 2021.
87. Lee CK, Man J, Lord S, Cooper W, Links M, Gebski V, Herbst RS, Gralla RJ, Mok T and Yang JC: Clinical and molecular characteristics associated with survival among patients treated with checkpoint inhibitors for advanced non-small cell lung carcinoma: A systematic review and meta-analysis. JAMA Oncol 4: 210-216, 2018.
88. Lee CK, Man J, Lord S, Links M, Gebski V, Mok T and Yang JCH: Checkpoint inhibitors in metastatic EGFR-mutated non-small cell lung cancer-a meta-analysis. J Thorac Oncol 12: 403-407, 2017.
89. Peters S, Gettinger S, Johnson ML, Jänne PA, Garassino MC, Christoph D, Toh CK, Rizvi NA, Chaft JE, Carcereny Costa E, *et al*: Phase II trial of atezolizumab as first-line or subsequent therapy for patients with programmed death-ligand 1-selected advanced non-small-cell lung cancer (BIRCH). J Clin Oncol 35: 2781-2789, 2017.
90. Garassino MC, Cho BC, Kim JH, Mazières J, Vansteenkiste J, Lena H, Corral Jaime J, Gray JE, Powderly J, Chouaid C, *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 19: 521-536, 2018.
91. Gubens MA, Sequist LV, Stevenson JP, Powell SF, Villaruz LC, Gadgeel SM, Langer CJ, Patnaik A, Borghaei H, Jalal SI, *et al*: Pembrolizumab in combination with ipilimumab as second-line or later therapy for advanced non-small-cell lung cancer: KEYNOTE-021 cohorts D and H. Lung Cancer 130: 59-66, 2019.
92. Yang JCH, Shepherd FA, Kim DW, Lee GW, Lee JS, Chang GC, Lee SS, Wei YF, Lee YG, Laus G, *et al*: Osimertinib Plus durvalumab versus osimertinib monotherapy in EGFR T790M-positive NSCLC following previous EGFR TKI therapy: CAURAL brief report. J Thorac Oncol 14: 933-939, 2019.
93. Jiang T, Wang P, Zhang J, Zhao Y, Zhou J, Fan Y, Shu Y, Liu X, Zhang H, He J, *et al*: Toripalimab plus chemotherapy as second-line treatment in previously EGFR-TKI treated patients with EGFR-mutant-advanced NSCLC: A multicenter phase-II trial. Signal Transduct Target Ther 6: 355, 2021.
94. Mok T, Nakagawa K, Park K, Ohe Y, Girard N, Kim HR, Wu YL, Gainor J, Lee SH, Chiu CH, *et al*: Nivolumab plus chemotherapy in epidermal growth factor receptor-mutated metastatic non-small-cell lung cancer after disease progression on epidermal growth factor receptor tyrosine kinase inhibitors: Final results of CheckMate 722. J Clin Oncol 42: 1252-1264, 2024.
95. Yang JCH, Lee DH, Lee JS, Fan Y, de Marinis F, Iwama E, Inoue T, Rodríguez-Cid J, Zhang L, Yang CT, *et al*: Phase III KEYNOTE-789 study of pemetrexed and platinum with or without pembrolizumab for tyrosine kinase inhibitor-resistant, EGFR-mutant, metastatic nonsquamous non-small cell lung cancer. J Clin Oncol 42: 4029-4039, 2024.
96. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, Rodríguez-Abreu D, Moro-Sibilot D, Thomas CA, Barlesi F, *et al*: Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N Engl J Med 378: 2288-2301, 2018.
97. Nogami N, Barlesi F, Socinski MA, Reck M, Thomas CA, Cappuzzo F, Mok TSK, Finley G, Aerts JG, Orlandi F, *et al*: IMpower150 final exploratory analyses for atezolizumab plus bevacizumab and chemotherapy in key NSCLC patient subgroups with EGFR mutations or metastases in the liver or brain. J Thorac Oncol 17: 309-323, 2022.
98. Lu S, Wu L, Jian H, Cheng Y, Wang Q, Fang J, Wang Z, Hu Y, Han L, Sun M, *et al*: Sintilimab plus chemotherapy for patients with EGFR-mutated non-squamous non-small-cell lung cancer with disease progression after EGFR tyrosine-kinase inhibitor therapy (ORIENT-31): Second interim analysis from a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Respir Med 11: 624-636, 2023.
99. Zhou C, Dong X, Chen G, Wang Z, Wu X, Yao Y, Zhang Y, Cheng Y, Pan H, Zhang X, *et al*: OA09.06 IMpower151: Phase III Study of Atezolizumab + bevacizumab + chemotherapy in 1L metastatic nonsquamous NSCLC. J Thorac Oncol 18 (Suppl): S64-S65, 2023.
100. Park S, Kim TM, Han JY, Lee GW, Shim BY, Lee YG, Kim SW, Kim IH, Lee S, Kim YJ, *et al*: Phase III, randomized study of atezolizumab plus bevacizumab and chemotherapy in patients with EGFR- or ALK-mutated non-small-cell lung cancer (ATTLAS, KCSG-LU19-04). J Clin Oncol 42: 1241-1251, 2024.
101. HARMONi-A Study Investigators, Fang W, Zhao Y, Luo Y, Yang R, Huang Y, He Z, Zhao H, Li M, Li K, *et al*: Ivonescimab plus chemotherapy in non-small cell lung cancer with EGFR variant: A randomized clinical trial. JAMA 332: 561-570, 2024.
102. Li Z, Fang W, Zhao Y, Luo Y, Yang R, Huang Y, He Z, Zhao H, Li M, Li K, *et al*: Ivonescimab combined with chemotherapy in patients with EGFR-mutant non-squamous non-small cell lung cancer who progressed on EGFR tyrosine-kinase inhibitor treatment (HARMONi-A): A randomized, double-blind, multi-center, phase 3 trial. J Clin Oncol 42 (16 Suppl): S8508, 2024.
103. Wu YL, Wang Z, Cheng Y, Fang J, Meng X, Pan Y, Zhao H, Zhao Y, Su H, Sun M, *et al*: 1255MO A phase II safety and efficacy study of PM8002/BNT327 in combination with chemotherapy in patients with EGFR-mutated non-small cell lung cancer (NSCLC). Ann Oncol 35 (Suppl 2): S804, 2024.

104. Fang WF, Yang Y, Zhao Y, Huang Y, Zhao H, Zhou N, Zhang Y, Chen L, Zhou T, Chen G, *et al*: 646P Iparomlimab and tuvonralimab (QL1706) plus chemotherapy and bevacizumab for epidermal growth factor receptor inhibitor (EGFRi)-resistant, EGFR-mutant, advanced non-small cell lung cancer (NSCLC): Updated results from Cohort 5 in the DUBHE-L-201 study. Ann Oncol 35 (Suppl 4): S1646, 2024.
105. Brown H, Vansteenkiste J, Nakagawa K, Cobo M, John T, Barker C, Kohlmann A, Todd A, Saggese M, Chmielecki J, *et al*: Programmed cell death ligand 1 expression in untreated EGFR mutated advanced NSCLC and response to osimertinib versus comparator in FLAURA. J Thorac Oncol 15: 138-143, 2020.
106. Lin C, Chen X, Li M, Liu J, Qi X, Yang W, Zhang H, Cai Z, Dai Y and Ouyang X: Programmed death-ligand 1 expression predicts tyrosine kinase inhibitor response and better prognosis in a cohort of patients with epidermal growth factor receptor mutation-positive lung adenocarcinoma. Clin Lung Cancer 16: e25-e35, 2015.
107. Soo RA, Kim HR, Asuncion BR, Fazreen Z, Omar MFM, Herrera MC, Yun Lim JS, Sia G, Soong R and Cho BC: Significance of immune checkpoint proteins in EGFR-mutant non-small cell lung cancer. Lung Cancer 105: 17-22, 2017.
108. Yoneshima Y, Ijichi K, Anai S, Ota K, Otsubo K, Iwama E, Tanaka K, Oda Y, Nakanishi Y and Okamoto I: PD-L1 expression in lung adenocarcinoma harboring EGFR mutations or ALK rearrangements. Lung Cancer 118: 36-40, 2018.
109. Su S, Dong ZY, Xie Z, Yan LX, Li YF, Su J, Liu SY, Yin K, Chen RL, Huang SM, *et al*: Strong programmed death ligand 1 expression predicts poor response and de novo resistance to EGFR tyrosine kinase inhibitors among NSCLC patients with EGFR mutation. J Thorac Oncol 13: 1668-1675, 2018.
110. Hsu KH, Huang YH, Tseng JS, Chen KC, Ku WH, Su KY, Chen JJW, Chen HW, Yu SL, Yang TY and Chang GC: High PD-L1 expression correlates with primary resistance to EGFR-TKIs in treatment naïve advanced EGFR-mutant lung adenocarcinoma patients. Lung Cancer 127: 37-43, 2019.
111. Sakata Y, Sakata S, Oya Y, Tamiya M, Suzuki H, Shibaki R, Okada A, Kobe H, Matsumoto H, Yokoi T, *et al*: Osimertinib as first-line treatment for advanced epidermal growth factor receptor mutation-positive non-small-cell lung cancer in a real-world setting (OSI-FACT). Eur J Cancer 159: 144-153, 2021.
112. Alves Pinto I, de Oliveira Cavagna R, Virginio da Silva AL, Dias JM, Santana IV, Souza LC, Ferreira da Silva FA, Biazotto Fernandes MF, Junqueira Pinto GD, Negreiros IS, *et al*: EGFR mutations and PD-L1 expression in early-stage non-small cell lung cancer: A real-world data from a single center in Brazil. Oncologist 27: e899-e907, 2022.
113. Papazyan T, Denis MG, Sagan C, Raimbourg J, Herbreteau G and Pons-Tostivint E: Impact of PD-L1 expression on the overall survival of caucasian patients with advanced EGFR-mutant NSCLC treated with frontline osimertinib. Target Oncol 19: 611-621, 2024.
114. Lakkunarajah S, Truong PT, Bone JN, Hughesman C, Yip S, Alex D, Hart J, Pollock P, Egli S, Clarkson M, *et al*: First-line osimertinib for patients with EGFR-mutated advanced non-small cell lung cancer: efficacy and safety during the COVID-19 pandemic. Transl Lung Cancer Res 12: 1454-1465, 2023.
115. Yoshimura A, Yamada T, Okuma Y, Fukuda A, Watanabe S, Nishioka N, Takeda T, Chihara Y, Takemoto S, Harada T, *et al*: Impact of tumor programmed death ligand-1 expression on osimertinib efficacy in untreated EGFR-mutated advanced non-small cell lung cancer: A prospective observational study. Transl Lung Cancer Res 10: 3582-3593, 2021.
116. Hsu KH, Tseng JS, Yang TY, Chen KC, Su KY, Yu SL, Chen JJW, Huang YH and Chang GC: PD-L1 strong expressions affect the clinical outcomes of osimertinib in treatment naïve advanced EGFR-mutant non-small cell lung cancer patients. Sci Rep 12: 9753, 2022.
117. Hamakawa Y, Agemi Y, Shiba A, Ikeda T, Higashi Y, Aga M, Miyazaki K, Taniguchi Y, Misumi Y, Nakamura Y, *et al*: Association of PD-L1 tumor proportion score ≥20% with early resistance to osimertinib in patients with EGFR-mutated NSCLC. Cancer Med 12: 17788-17797, 2023.