Role of the dynamic tumor microenvironment in controversies regarding immune checkpoint inhibitors for the treatment of non-small cell lung cancer (NSCLC) with EGFR mutations

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Abstract

Immunotherapy has been incorporated into the first- and second-line treatment strategies for non-small cell lung cancer (NSCLC), profoundly ushering in a new treatment landscape. However, both adaptive signaling and oncogenic (epidermal growth factor receptor (EGFR)-driven) signaling may induce PD-L1 upregulation in NSCLC. Nevertheless, the superiority of immune checkpoint inhibitors (ICIs) in advanced EGFR-mutant NSCLC is only moderate. ICIs appear to be well tolerated, but clinical activity for some advanced EGFR-mutant NSCLC patients has only been observed in a small proportion of trials. Hence, there are still several open questions about PD-L1 axis inhibitors in patients with NSCLC whose tumors harbor EGFR mutations, such as the effect of EGFR tyrosine kinase inhibitors (TKIs) or EGFR mutations in the tumor microenvironment (TME). Finding the answers to these questions requires ongoing trials and preclinical studies to identify the mechanisms explaining this possible increased susceptibility and to identify prognostic molecular and clinical markers that may predict benefits with PD-1 axis inhibition in this specific NSCLC subpopulation. The presence of multiple mechanisms, including dynamic immune TME profiles, changes in PD-L1 expression and low tumor mutational burdens, may explain the conflicting data regarding the correlation between PD-L1 axis inhibitors and EGFR mutation status. We conducted a review of this currently controversial topic in an attempt to aid in the decision-making process.

Keywords: Tumor microenvironment, EGFR mutations, Non-small cell lung cancer, Immunotherapy, Anti-PD-1/PD-L1 treatment

Introduction

Lung cancer is the most common malignant tumor (11.6%) and the leading cause of cancer-related death (18.4%) worldwide [1]. According to the latest International Agency for Research on Cancer (IARC) report, there are approximately 2.1 million lung cancer patients worldwide. In 2018, there were an estimated 2,093,876 new cases of lung cancer worldwide and approximately 1,761,007 deaths [1]. Eighty percent of new lung cancer patients are diagnosed with NSCLC [2], which does not have obvious clinical results and/or symptoms in the early stage. When patients are diagnosed with NSCLC, the optimal treatment period is often missed. Seventy-five percent of NSCLC is diagnosed at an advanced stage, resulting in a 5-year survival rate of less than 15% [3, 4].

Recent advances in next-generation sequencing (NGS), other high-throughput genomic profiling platforms and the generation of multiple genetically engineered mouse models (GEMMs) of lung cancer have allowed researchers to transform the view of NSCLC from histopathological descriptions to precise molecular and genetic identities that can be resolved at the single-cell level [5]. Following the identification of KRAS and BRAF mutations, EGFR mutations were discovered in patients with lung...
adenocarcinoma (ADC) and were associated with the response to EGFR inhibitors. Given this relatively large number of mutations per tumor, the treatment of NSCLC has entered a new revolutionized era of molecular targeted therapy [5]. Currently, EGFR tyrosine kinase inhibitors (EGFR-TKIs) are recommended by clinical guidelines as first-line therapeutic drugs [6–8] for advanced NSCLC patients with EGFR-sensitive mutations and no resistance genes. Compared to chemotherapy, EGFR-TKIs have demonstrated superior survival [9] in terms of the objective response rate (ORR) (67.0% vs 40.8%) and median progression-free survival (PFS) (10.9 months vs 7.4 months). However, these compounds have provided only initial improvement in clinical outcomes, and acquired resistance within 9–14 months is almost inevitable [10–12]. An innovative treatment for overcoming EGFR-TKI resistance remains an unresolved issue. This topic has gained increasing attention for strengthening the potential benefits of immunotherapy [13]. ICIs have already shown excellent survival benefits for NSCLC patients with long-term efficacy and less toxicity [14–22]. For example, the longest follow-up analysis of data from a clinical trial showed that 129 patients with advanced NSCLC who had failed multiple treatments had a 5-year survival rate of 26% after receiving nivolumab [16], which was much higher than the 5-year survival rate of 1–8% in NSCLC patients who did not receive ICIs [23, 24]. Furthermore, preclinical results have shown that EGFR activation can upregulate intrinsic PD-L1 expression on tumor cells, which induces T cell apoptosis and contributes to the immune escape of EGFR-mutant NSCLC. In addition, EGFR-TKIs can potentiate the induction of MHC class I and II molecules in response to IFN-γ and enhance T cell-mediated tumor killing [47]. In this regard, these studies provide a theoretical basis to support the potential synergistic effects of combining PD-1/PD-L1 inhibitors and EGFR-targeted therapy in NSCLC patients carrying EGFR mutations accompanied by upregulation of PD-L1 expression.

A number of related studies to evaluate the safety and efficacy of immunotherapy combined with targeted therapy in patients with EGFR mutations are currently underway. Most recent clinical trials have shown that patients with EGFR mutations are unable to benefit from immunotherapy. Intriguingly, immunotherapy in these patients may be associated with the development of hyperprogressive disease (HPD) and lead to increased toxic effects [25, 26]. The CAURAL trial is a multiphase III trial in which osimertinib is combined with durvalumab. Both EGFR-TKI-sensitizing- and EGFR T790 M mutation-positive advanced patients were included in the study, though the results did not show a benefit for the combination arms with regard to ORR (64% vs 80%), duration of response (DOR) (17.5 months vs 21.4 months) or disease control rate (DCR) (93% vs 100%), which was even lower than that of the osimertinib monotherapy treatment group [27]. In addition, the KEYNOTE-021 study [28] was conducted to test the efficacy of combination pembrolizumab with erlotinib in EGFR-mutant advanced NSCLC patients. The combination treatment enhanced the median PFS (19.5 months) benefit along with ORR (41.7%) compared with that of patients taking first-generation EGFR-TKIs (11.0 months) or osimertinib (19.2 months) [29]. Moreover, preliminary results from other early studies have shown promising efficacy and acceptable toxicity. Specifically, in the phase I study of nivolumab (CheckMate 012), 21 EGFR-mutant NSCLC patients were treated with the combination of nivolumab and erlotinib associated with an acceptable toxicity profile, with a 15% ORR, 65% DCR, 5.1-month median PFS and 18.7-month median overall survival (OS) [30]. This trial also reported one TKI-naïve patient who was effectively treated with nivolumab plus erlotinib, with an ongoing response lasting more than 5 years. However, others have demonstrated the opposite result (Table 1). Overall, the combined use of PD-1/PD-L1 inhibitors and EGFR-TKIs remains controversial.

Recent preclinical and clinical studies have begun to reveal limited benefit of immune checkpoint inhibitors in EGFR-mutant NSCLC patients. Several reports have reported that the tumor microenvironment (TME) [31–36], tumor immunogenicity [37–39], tumor-specific mutations, copy number variants [40, 41] and abundances of specific intestinal bacteria can [42] affect the efficacy of ICIs. Multiple studies have demonstrated that EGFR mutations in NSCLC are more likely to correlate with an immunosuppressive TME [41, 43–48], the tumor mutation burden (TMB) [43, 49], and expression of PD-L1 [41, 50–53]. In addition, EGFR-TKIs may modulate the immune response by regulating TME. These factors are continuous variables in space and time, and the exact boundaries and correlations among them are still unclear [54]. The above findings might explain the contradictory clinical results for ICIs combined with EGFR-TKIs among patients with newly diagnosed or treated EGFR-mutant NSCLC.

In this review, we endeavor to compare and analyze all preclinical and clinical studies on the feasibility of treatment with ICIs or combined with EGFR-TKIs in NSCLC patients with EGFR mutations. We explore the unique TME of these patients, which may cause an inferior response to ICIs. We critically discuss the mechanisms underlying contradictory results in monotherapy and combination therapy and focus on improving the effectiveness of immunotherapy in EGFR-mutant NSCLC. Additional studies are warranted to further discover and identify prognostic biomarkers in patients with advanced EGFR-mutant NSCLC and to predict the benefits of anti-PD-1/PD-L1 treatment in this special NSCLC subpopulation.
| Clinical Trial | Number of patients | Median age (yr) | Male Sex (%) | Baseline | Treatment | ORR (%) | Median OS (months) | Median PFS (months) | Phase | Status |
|----------------|--------------------|----------------|--------------|----------|-----------|---------|-------------------|-------------------|-------|--------|
| NCT02088112 [188] | 10 | NA | NA | TKI-naive EGFR (+) | Concurrent Gefitinib+Durvalumab | 77.8 | NA | NA | 1 | Active/not recruiting |
| NCT02040064/GEFTBM [189] | 18 | 66 | 35 | TKI-pretreated EGFR (+) | Gefitinib + Tremelimumab | 50–80 | NA | NA | 1 | Completed |
| NCT02574078/CheckMate 370 [190] | NA | NA | NA | TKI-naive EGFR (+) | Nivolumab + Erlotinib | NA | NA | NA | 1/2 | Active/not recruiting |
| NCT02013219 [192] | 28 | 61 | NA | TKI-naive EGFR (+) and treatment-naive ALK (+) | Atezolizumab+Erlotinib | 75 | NA | 11.3 | 1 | Active/not recruiting |
| NCT01454102/CheckMate 012 [191] | 21 | NA | NA | TKI-naive EGFR (+) | Pembrolizumab+Erlotinib | 41.7 | 2 | 19.5 | 1/2 | Active/not recruiting |
| NCT0209674/KEYNOTE-021 [27] | 12 | 60 | 50 | TKI-naive EGFR (+) | Pembrolizumab+Erlotinib | 14.3 | 3 | 1.4 | 1 | Active/not recruiting |
| NCT01642004/CheckMate-017 [15] | 135 | 62 | 82 | Platinum-Based Chemotherapy Pretreated | Nivolumab | 20 | 92 | 3.5 | 3 | Active/not recruiting |
| NCT01905657/Keynote-010 [17] | 345 | 63 | 62 | Platinum-Based Chemotherapy Pretreated | Pembrolizumab | 9–18 | 85–12.7 | 4 | 2/3 | Active/not recruiting |
| NCT02082277/OAK [18] | 425 | 63 | 61 | Platinum-Based Chemotherapy Pretreated | Atezolizumab | 14 | 138 | 2.8 | 3 | Completed |
| NCT01903993 /POPLAR [19] | 144 | 62 | 65 | Platinum-Based Chemotherapy Pretreated | Atezolizumab | 14 | 126 | 2.7 | 2 | Completed |
| NCT02087423/ATLANTIC [194] | 111 | 61 | NA | Pretreated-EGFR (+)/ALK(+) | Durvalumab | NA | 133 | NA | 2 | Active/not recruiting |
| Clinical Trial | Number of patients | Median age (yr) | Male Sex (%) | Baseline Treatment ORR (%) | Median OS (months) | Median PFS (months) | Phase Status |
|---------------|-------------------|----------------|--------------|-----------------|-------------------|-------------------|--------------|
| NCT01295827/Keynote-001 [180] | 68 | 61 | NA | Pretreated- EGFR (-)/ALK(-) | Durvalumab | NA | 13.2 | NA | 2 | Active: not recruiting |
| NCT02366143/IMpower150 [187] | 45 | 63 | 38 | Chemotherapy-naiveEGFR (+) | Atezolizumab+Carboplatin+ Paclitaxel | 36 | 21.4 | 6.9 | 2 | Active, not recruiting |
| NCT02367781/IMpower130 [195] | 32 | NA | 21 | EGFR (+)/ALK(+) | Atezolizumab + Carboplatin + Paclitaxel + Bevacizumab | NA | 144 | 7.0 | 2 | Active, not recruiting |
| NCT02367781/IMpower130 [195] | 12 | NA | NA | Carboplatin + Paclitaxel | NA | 100 | 6.0 | 2 | |

EGFR Epidermal growth factor receptor, OS Overall survival, PFS Progression-free survival, ORR Objective Response Rate, ALK Anaplastic lymphoma kinase, EGFR-TKI Epidermal growth factor receptor-tyrosine kinase inhibitor, NA Not available, NR Not reached, NE Not estimable, NSCLC Non-small cell lung cancer, PD-L1 Programmed death-ligand 1

*24-week PFS rate was 51% 
1Received ≥2 prior systemic treatment regimens + ≥ 90% of TC expressing PD-L1
EGFR mutations affect the efficacy of anti-PD-1/PD-L1 treatment

Several studies have demonstrated the possible poor efficacy of PD-1 inhibitors for treating EGFR-mutant NSCLC patients [14, 40, 51]. Two meta-analyses on the efficacy of ICIs versus docetaxel in patients with pre-treated advanced NSCLC have been recently reported [39, 50]. In a report by Lee [50], there was a 32% reduction in the risk of death with ICIs compared with docetaxel in the intention to treat population (HR = 0.68, 95% CI: 0.61–0.77, P < 0.0001). Checkpoint inhibitors prolonged OS in the wildtype EGFR subgroup (HR = 0.66, 95% CI: 0.58–0.76, P < 0.0001) but not in the mutant EGFR subgroup (HR = 1.05, 95% CI: 0.70–1.55, P = 0.81). A similar analysis confirmed that ICIs do not enhance OS in NSCLC patients with EGFR mutations compared with that in patients taking docetaxel (HR = 1.09, 95% CI: 0.84–1.41) [39]. Another meta-analysis covering five clinical trials (Checkmate 017 and 057, Keynote 010, OAK, POPLAR) [14] also verified that patients with EGFR-sensitive mutations dramatically responded to docetaxel compared with PD-1 inhibitors (HR = 0.69, 95% CI:0.63–0.75; P < 0.001). Thus, a key question remains as to whether the benefit of ICIs among NSCLC patients with EGFR mutations is limited.

Underlying mechanisms for the poor efficacy of anti-PD-1/PD-L1 treatment in EGFR-mutant NSCLC

EGFR mutations affect the TME in NSCLC

The TME is the internal environment in which tumor cells depend on survival and development. TME is critical for the development of tumor immunotherapy strategies, and T lymphocytes, myeloid cells, cytokines, and exosomes constitute the immune regulatory networks [55, 56] of the TME. With tumor development and the plasticity of immune cells, T lymphocytes switch from having immune surveillance to immune escape [57, 58] functions via immunoeediting and even exhibit immunosuppressive functions such as inducing regulatory T (Tregs) cells and upregulating myeloid-derived suppressor cells (MDSCs) [57, 59–63]. In addition, inflammatory cells and immunomodulatory mediators in the TME may be involved in an important mechanism to mediate tumor progression [60, 61, 64].

Immunosuppressive effects of EGFR mutations have also been described in recent years. Several studies have reported that EGFR mutations can modulate possible factors related to the status of the TME, such as tumor-infiltrating lymphocytes (TILs) [41, 43, 65], Tregs [45, 66], MDSCs [47, 67] tumor-associated macrophages (TAMs) [47], immunoregulatory cytokines [47, 48] and exosomes [68]. These preclinical and clinical findings suggest that the TME of NSCLC patients with EGFR mutations may be unique, differing from patients with wildtype EGFR, and that EGFR mutations may impact the antitumor immune response by affecting the TME (Fig. 1).

EGFR mutations and regulatory T cells (Tregs)

Tregs are regarded as a critical hurdle in antitumor immunity, and the transcriptional factor Foxp3 serves as a lineage specification factor for Tregs [69, 70]. TGF-β, IL-10 and IL-35 secreted by Tregs in tumors can produce an immunosuppressive environment that actively attenuates and subverts the antitumor immune responses of CD4+ T cells, CD8+ T cells and natural killer (NK) cells [71–74].

Huang et al. [66] showed that EGFR-containing exosomes induce the plasticity transformation of tolerant DCs and cause DCs to produce indoleamine 2, 3-dioxygenase (IDO), which plays an important role in converting CD3 + CD4 + CD25+ T cells into Tregs; these results suggest that IDO expression can upregulate Treg function and induce immune tolerance and evasion [75, 76]. In addition, studies have shown that amphiregulin (AREG) is one of the EGFR ligands, and its level of plasma expression in patients with NSCLC is associated with a poor prognosis [77]; moreover, as a specific molecule in exosomes of tumor cells, AREG plays a role in promoting tumor progression [78]. Wang et al. [79] found that AREG mediated Treg suppressive function via the EGFR/GSK-3/Foxp3 axis in vitro and in vivo. Furthermore, inhibition of EGFR by the EGFR-TKI gefitinib restored the activity of GSK-3β and attenuated Treg function [80]. Mascia et al. [45] also showed that knockdown of the EGFR gene significantly inhibited tumor cell growth and downregulated Treg infiltration in the TME.

EGFR mutations and tumor-infiltrating lymphocytes (TILs)

TILs are a group of tumor-infiltrating and antigenic cell populations that can exist in tumor cancer nests and stroma [81]. CD8+ T cells act as antitumor immune cells during the development of the TME and destroy malignant T cells by releasing cytokines such as IFN-γ, perforin and granzyme B; the amount of CD8+ T cells determines the efficiency of tumor cell killing. Multiple studies have shown that highly infiltrating CD8+ TILs in NSCLC are associated with a good prognosis and good treatment efficacy [31, 82–84]. Teng et al. [85] evaluated the efficacy of immunotherapy by establishing a TME model based on the expression of TIL and PD-L1, suggesting that the immunoinflammatory TME (PD-L1+ and TIL+) is most likely to benefit from anti-PD-1/PD-L1 treatments and that lower levels of CD8+ TILs are associated with EGFR mutations [41, 43, 65, 86].

Dong et al. [43] found significantly reduced CD8+ TILs in an EGFR-mutant group compared with a wildtype EGFR group (P = 0.003). Notably, a significant difference in PD-L1 and CD8+ TIL combined expression between EGFR mutations showed a significantly lower
Fig. 1 (See legend on next page.)
ratio of PD-L1+/TIL+ but a higher ratio of PD-L1−/TIL− than the EGFR wildtype group (odds ratio (OR): 1.79, 95% CI: 1.10−2.93; \( P = 0.02 \)). Investigators also found a significant difference in EGFR mutations between the PD-L1−/TIL− group and the PD-L1+/TIL+ group (\( P = 0.005 \)), and patients with low PD-L1+/TIL+ carried EGFR mutations. Quantitative fluorescence images revealed TIL activation through identification of Ki67 (proliferation of T cells) and granzyme B (cytotoxic activity of TILs) in CD3+ cells [87]. Toki et al. [88] used fluorescence to explore the association between EGFR mutations and TIL status. An exhausted or dormant immune status (high CD3 with low Ki67 and low granzyme B) was detected in 28.6% (16/56) of those in the mutant EGFR group, and tumor cells and stromal cells with high PD-L1 expression were more likely to have highly infiltrating activated TILs (\( P = 0.0014 \) and \( P = 0.02 \), respectively). In addition, differences in immunological profiles according to EGFR mutation sites have been reported: the prevalence of the inflammatory TME, such as significantly higher expression of CD8+ T cells (\( P = 0.03 \)) in EGFR L858R samples as well as a trend of higher levels of CD3+ and CD4+ T cells (\( P = 0.11 \) and \( P = 0.11 \), respectively) than in EGFR exon 19 deletion samples was observed; a higher level of infiltrating functional TILs was noted in the EGFR L858R group, but without differences in PD-L1 expression in tumor cells and stromal cells [88, 89].

**EGFR mutations and exosomes**

Exosomes are small membrane vesicles that are secreted by cells and contain many molecules, such as nucleic acids, lipids and proteins [89]. Exosomes act as a signal carrier to mediate cell-to-cell communication and affect the sensitivity of tumor cells to drugs, which is associated with the occurrence of tumor metastasis [72, 73, 90–96]. Tumor cell-derived exosomes can affect distant target cells through their intrinsic miRNAs, alter the local microenvironment, and form a pretransfer endometrium to exert remote regulatory functions [97]. Poggi et al. [68] reported that tumor cells secrete exosomes harboring PD-L1, leading to immune escape via direct binding to T cells and inhibition of their function. In addition, PD-L1-carrying exosomes are able to inhibit the activity of T cells in lymph nodes. It was also found that PD-L1 in exosomes is resistant to PD-L1 inhibitors and that knockout by clustered regularly interspaced short palindromic repeats (CRISPR) of genes related to exosomes can cause systemic antitumor immunity and immune memory and have significant effects after immunotherapy. In addition, the combined inhibition of exosome formation and anti-PD-1/PD-L1 treatment resulted in significantly longer survival time in mice compared to mice receiving monotherapy [68].

**Cell surface molecules and selected soluble factors**

Changes in expression of membranous immunomodulatory molecules in the TME and release of immunosuppressive soluble factors, such as TGF-β, IL-10 and adenosine (ADO) [47, 48], play a crucial role in tumor progression.

**EGFR mutations and CD73**

CD73 is an extracellular 5′-nucleotidase anchored to cell membrane lipid rafts by glycosylphosphatidylinositol (GPI), which is highly expressed in various tumors. CD73 is not only involved in purine and pyrimidine nucleotide synthesis and salvage pathways but is also an important negative regulator of immune signaling involved in the immune escape
of tumors by catalyzing the formation of ADO, which is an immunosuppressive medium [98]. Studies have shown that high expression of CD73 is associated with both immunosuppression and poor prognosis in patients with NSCLC [98–100]. Therefore, understanding the crosstalk between CD73, ADO and TME is an area of active research, as described below (Fig. 1).

Park et al. [48] stratified data according to CD73 expression levels [CD73 high expression (CD73-H) and CD73 low expression (CD73-L)] and found that compared with the CD73-L group, the CD73-H group was less likely to have high-density infiltrating activated CD4+ T cells (20% vs 41%, \( P < 0.01 \)) and CD8+ T cells (28% vs 47%, \( P < 0.01 \)). The OS and median disease-free survival (DFS) were higher in the CD73-L group compared to the CD73-H group (62 vs 44 months, \( P < 0.01 \); 83 vs 34 months, \( P < 0.01 \)), and subgroup findings suggested an association between EGFR mutations and higher CD73 expression (\( P = 0.03 \)) [48]. Therefore, Park et al. hypothesized that overexpression of CD73 in mutant EGFR NSCLC may result in a poor response to immunosuppressive therapy and suggested that the combination of CD73 inhibitors with EGFR-TKIs or PD-1/PD-L1 inhibitors may be a potential strategy for treating drug-resistant patients [99]. In contrast, a retrospective study reported that CD73 overexpression compared to low CD73 expression in an EGFR-TKI-resistant group treated with immunotherapy resulted in a longer median PFS (16 months vs 1.2 months, \( P = 0.024 \)) and ORR (66.7% vs 0%, \( P = 0.006 \)), with no difference between the high and low CD73 expression groups of wildtype EGFR patients (median PFS: 2.8 months vs 2.8 months, \( P = 0.394 \)) [101]. However, the current consensus suggests that EGFR-mutant tumor cells may upregulate CD73, convert ATP to ADO, upregulate expression of Tregs through ADO bypass, and change the function of tumor cells and immune cells, resulting in an immunosuppressive TME. Due to inconsistent results, the precise mechanism by which CD73 expression is associated with an immunosuppressive TME remains unclear.

**EGFR mutations and major histocompatibility complex (MHC)** MHC plays an important role in tumor antigen presentation. MHC class I molecular tumor antigens constitute the first signal of cell activation, activate CD8+ T cells, and exert antitumor immune effects. MHC class II molecules bind to tumor antigen peptides and are presented to CD4+ T cells, which activate specific CD4+ T cells. The former can specifically kill tumor cells, and the latter participate in the body’s antitumor positive feedback regulation [102] by secreting cytokines to enhance the cell-killing effect. It has been previously reported that expression of MHCI and/or MHCII molecules can impact the antitumor immune response [103–105]. IFN-\( \gamma \) potentiates the induction of MHC class I (MHCI) and II (MHCII) molecules [102, 106]. Watanabe et al. found that EGFR-mutant cells have lower levels of human leukocyte antigen (HLA)-B expression than do EGFR-wildtype cells [107] in the presence of IFN-\( \gamma \). Additionally, several recent studies report that MHC-I and MHC-II expression is downregulated via the IFN-\( \gamma \) signaling pathway and downstream MEK/ERK signaling pathways (Fig. 2) [102, 107–111].

**EGFR gene mutation sites and the effectiveness of immune checkpoint inhibitors** EGFR gene mutations mainly occur in exons 18–20; the exon19 deletion mutation (p. E746-A750del) and exon 21 point mutations (p.L858R) account for more than 85% of all mutation types. These two mutations are also the two [112] that result in greatest sensitivity to EGFR-TKIs. All other mutations can be referred to as uncommon mutations. The G719X mutation in exon 18 (3%) [113] and the L861X mutation in exon 21 (2%) [114] are the most common uncommon mutation types; G719X, L861X, and additional mutations in exon 20 and exon 19 are also considered favorable for effective EGFR-TKI treatment [115–117]. Recently, multiple studies have shown that NSCLC patients with uncommon EGFR mutations are more likely to benefit from immunotherapy than are those with common EGFR mutations [118, 119].

Yamada et al. [118] reported that NSCLC patients with uncommon EGFR mutations showed a good response to ICIs compared with patients with common EGFR mutations, with prolonged median PFS (256 days vs 50 days, \( P = 0.003 \)) and median time to progression (TTP) (256 days vs 48 days, \( P = 0.008 \)). A 5-year follow-up of the CA209–003 study on nivolumab, a phase I single-arm study, was reported by Brahmer and colleagues [120], who observed that 2 patients with uncommon EGFR mutations (2/8, 25%) had a survival of more than 5 years; the mutations were the EGFR exon 20 insertion and exon 18 missense mutation G719A. Thus far, long-term efficacy and safety data for ICIs from randomized trials have been lacking for NSCLC. Additionally, in a report by Yoshida and colleagues [119], a longer PFS rate was observed in patients with uncommon EGFR mutations treated with nivolumab than in those with common EGFR mutations (\( P < 0.05 \)) [47].

**EGFR mutations and tumor mutation burden (TMB)** The TMB is the total number of substitution, insertion, and deletion mutations per megabase of the coding region of a tumor gene; it is a good biomarker for predicting the efficacy of immunotherapy and can quantitatively estimate the total number of mutations in the coding region of the tumor genome. A higher TMB is related to more new antigens produced by tumors, easier recognition by immune cells and a long-lasting clinical response [38].
Notably, compared to an EGFR-resistant/unknown group, a significantly lower TMB was found for EGFR-sensitive mutations (defined based on response to first-generation EGFR-TKIs). Haratani et al. [86] evaluated the efficacy of nivolumab with regard to TMB in NSCLC patients with mutant EGFR; a median TMB of 101 was reported for each tumor patient, and patients who dramatically responded to nivolumab had a significantly higher TMB than did non-responders. In addition, Dong et al. [43] found a significantly reduced median TMB in an EGFR-mutant group (exons19Del, L858R, L861Q, G719X, and S768I) compared with an EGFR-wildtype group (56 vs 181). They also found that the median ratio of the EGFR mutant to wildtype TMB was 59:209 (Broad data set) and 162:197 (GLCI data set). Mutations such as those in EGFR, BRAF and TP53 have been shown to typically occur as early clonal, initiating drivers. Current studies show that most patients with specific gene mutations do not respond well to immunotherapy, and reduced TMB may be a mechanism for a poor response to ICIs in patients with EGFR mutation [37, 43, 49]. Attempts to establish a correlation between EGFR mutations and TMB are ongoing, and the role of microsatellite instability-high (MSI-H) and mismatch-repair deficiency (dMMR) in immunotherapy also needs to be explored.

EGFR mutations and PD-L1 expression

The regulation of EGFR mutations and PD-L1 expression remains controversial, but experimental data [121–124] indicate that EGFR mutations directly or indirectly drive PD-L1 upregulation in NSCLC cells (co-cultured with immune cells). In addition, there is some evidence to support that activation of downstream EGFR signaling [124–134], such as Ras/RAF/MEK/ERK, PI3K/AKT/mTOR, JAK/STAT, NF-kB and GSK-3β, leads to PD-L1 expression (Fig. 2). However, this “intrinsic” mechanism of PD-L1 upregulation is contrasted by several recent clinical studies concluding that PD-L1 is highly expressed in EGFR-wildtype NSCLC [135], and there is a negative correlation [43, 136–139] or no significant correlation [135] between EGFR mutations and PD-L1 expression. One meta-analysis [139] revealed lower PD-L1 expression rates in EGFR-mutant than in EGFR-wildtype tumors (36.7% vs 44.1%, \( P < 0.05 \)). Another pooled analysis [43] had the same conclusion: expression of PD-L1 in EGFR-wildtype tumors was significantly higher than that in EGFR-mutant tumors (\( P = 0.02 \)). For further confirmation, the researchers examined mRNA profiles, PD-L1 immunohistochemistry (IHC) and reverse-phase protein arrays (RPPAs) of tumor samples and found that expression of PD-L1 in EGFR-mutant
tumors was significantly lower than that in the EGFR-wildtype tumors ($P < 0.05$). Nonetheless, others have reported the opposite results [51, 122].

Such inconsistent results of different experimental studies might be associated with various factors, such as different PD-L1 detection techniques (different antibodies, detection platforms, and different set positive thresholds), tumor heterogeneity, and patient tumor tissue sources (such as cytological specimens, archived specimens, fresh specimens, primary and metastasis sites). Additionally, increased expression of PD-L1 on immune cells can induce immune escape. TILs can also be used to detect PD-L1 expression [20, 140–145]. Noguchi et al. [146] reported that expression of PD-L1 on TAMs might have an important role in tumor immune escape. Induction of PD-L1 on tumor cells is regulated by two major pathways: one driven by IFN-$\gamma$ and another controlled by constitutive oncogenic signaling. However, as expression of PD-L1 on immune cells is pronounced, only partially dependent on IFN-$\gamma$, and is relatively stable during monitoring, Noguchi et al. concluded that expression of PD-L1 on immune cells is a good biomarker [146].

**T790 M mutation status and the effectiveness of immune checkpoint inhibitors**

An EGFR-sensitive mutation group (defined based on response to first-generation EGFR-TKIs) exhibited a significantly longer PFS compared to an EGFR-resistant/unknown group [147–150]. However, acquired resistance to EGFR-TKIs develops after 9–14 months, and approximately 50–60% of such resistance is mediated by T790M [151]. Haratani et al. [86] observed a benefit of nivolumab in T790M(−) patients compared with T790M(+) patients. Additionally, T790M(−) patients had significantly lower FOXP3+ TILs than did T790M(+) patients ($P = 0.013$). Furthermore, in a retrospective study by Yamada et al. [118] including 27 patients with EGFR-TKI resistance who were treated with ICIs, subgroup findings supported that T790M(−) patients were more likely to derive greater benefit from PD-1 inhibitor treatment (median PFS: 86 days vs 48 days, $P = 0.03$; median TTP: 97 days vs 48 days, $P = 0.03$) than were T790M(+) patients. In the study of 67 EGFR-mutant NSCLC patients, the prevalence of PD-L1 expression was significantly lower in T790M(+) tumors than in T790M(−) tumors ($P = 0.0149$), and better survival was associated with PD-L1(−) / T790M(+) tumors [152].

**Immune modulatory effects of EGFR-TKIs**

**EGFR-TKIs affect the TME in NSCLC**

To date, preclinical and clinical studies have shown that EGFR-TKIs can induce antitumor immunity through the following [45, 79, 102, 107, 108, 153–156]: potentiating induction of class I (MHCI) and II (MHCII) molecules; promoting Foxp3 degradation to attenuate the inhibitory function of Tregs; reducing the infiltration of Tregs in the TME and inhibiting tumor growth; and enhancing the cytotoxicity of cytotoxic T lymphocytes (CTLs) that mediate antitumor immune response, reduce T cell apoptosis, and increase IFN-$\gamma$ secretion to enhance the immune system response. Thus, EGFR-TKIs show promising efficacy for anti-PD-1/PD-L1 treatment. However, these possible mechanisms regarding the immunostimulatory effect of EGFR-TKIs do not fully explain the controversial results of the combination of ICIs and EGFR-TKIs in patients with EGFR mutations [27, 28, 30].

Using a murine model, Jia et al. [47] recently observed a dynamic effect of EGFR-TKIs on the tumor immune microenvironment from beneficial (early treatment) to immunosuppressive (later treatment) (Fig. 1; Table 2). The short-term inhibition of tumor cell growth early in EGFR-TKI treatment is obvious, including an increase in the number of CD8+ T cells, DCs and M1-like TAMs, a decrease in Treg infiltration and inhibition of M1-like TAMs to M2-like TAMs. Jia and colleagues also reported that certain immunosuppressive factors accumulate gradually throughout treatment. However, later in EGFR-TKI treatment, they found there was either no significant change or even a decrease in antitumor effector cells and increasing secretion of IL-10 and CCL2 in serum. CCL2, a key effector cytokine with expression that is upregulated by EGFR-TKIs, plays an important role in the migration of MDSCs to the TME [157–160]. CCL2 induces T cells to differentiate to Th2 cells (anti-inflammatory function), which upregulate and activate the signal transducer and transcriptional activator-3 (STAT3) pathway of MDSCs. Thus, STAT3 further mediates the amplification and activation of MDSCs [161], exerting antitumor immunosuppressive effects [162], such as producing the immunosuppressive molecules IL-10 and TGF-β, inhibiting antitumor functions [163–168], inducing T cell apoptosis [169], upregulating Tregs [170] and promoting M2 phenotype polarization in TAMs [171]. In addition to suppressing immune responses, MDSCs are associated with tumorigenesis by promoting metastasis and inducing angiogenesis, including the secretion of vascular endothelial growth factor (VEGF), direct differentiation into tumor vascular endothelial cells [172], and release of matrix metalloproteinase (MMP) [173, 174]. Investigators have also reported that IL-10 [175] not only mediates immature myeloid cells (IMCs) via the STAT3 pathway to activate MDSCs
but also suppresses HLA class expression on the tumor cell surface. Thus, according to the data by Jia and colleagues, there may be a small window of immune microenvironmental changes in which EGFR blockade is most beneficial in the setting of combinations with immune-mediated anticancer approaches. One key question is whether a similar phenomenon occurs in EGFR-TKI-treated NSCLC, which is critical to improve the efficacy of immunotherapy combined with targeted therapy.

EGFR-TKIs cause dynamic changes in expression of PD-L1
An intriguing phenomenon occurs in EGFR-TKI-treated NSCLC, which is critical to improve the efficacy of immunotherapy combined with targeted therapy.

Table 2 The Dynamic Changes Occurring in the Immune Microenvironment after EGFR Inhibition in an EGFR-Mutant Transgenic Mouse Model

| Effect of EGFR-TKIs on tumor immune Microenvironment | Early Treatment | Later Treatment | Throughout Treatment |
|-----------------------------------------------------|-----------------|-----------------|----------------------|
| Tumor burden ↓ | Tumor burden ↓ | Tumor burden ↓ |
| Tumor size ↓  | Tumor size ↓  | Tumor size ↓  |
| PD-L1↑        | PD-L1↑        | PD-L1↑        |
| PD-1↓         | PD-1↓         | PD-1↓         |
| CTLA-4↑       | CTLA-4↑       | CTLA-4↑       |
| TIM-3↑        | TIM-3↑        | TIM-3↑        |
| CD3+ lymphocytes↑ | CD3+ lymphocytes↑ | CD3+ lymphocytes↑ |
| CD8+ T cells↑ | CD8+ T cells↑ | CD8+ T cells↑ |
| Foxp3+ Tregs↓ | Foxp3+ Tregs↓ | Foxp3+ Tregs↓ |
| CD11b + Myeloid Cells↑ | CD11b + Myeloid Cells↑ | CD11b + Myeloid Cells↑ |
| PMN-MDSCs +/− | PMN-MDSCs +/− | PMN-MDSCs +/− |
| M-MDSCs↑ | M-MDSCs↑ | M-MDSCs↑ |
| DCs↑ | DCs↑ | DCs↑ |
| M1-TAM↑ | M1-TAM↑ | M1-TAM↑ |
| M2-TAM↓ | M2-TAM↓ | M2-TAM↓ |
| IL-10 +/− | IL-10 +/− | IL-10 +/− |
| CCL-2 +/− | CCL-2 +/− | CCL-2 +/− |

**PD-L1 Programmed death-ligand 1, PD-1 Programmed cell death protein 1, CTLA-4 The cytotoxic T-lymphocyte-associated antigen 4, TIM-3 T-cell immunoglobulin and mucin-domain containing 3, PMN-MDSC Polyomorphonuclear MDSCs, M-MDSC Mononuclear MDSCs, IL-10 Interleukin 10, CCL-2 The chemokine (C-C motif) ligand 2**

**a:** PD-L1 expression after EGFR-TKI monotherapy in both CD45+ immune cells and CD4− tumor cells;

**b:** PD-1, CTLA-4, and TIM-3 expression on CD3+ lymphocytes;

+/−: Remained unchanged
↑: increased
↓: decreased

NSCLC specimens was associated with a significant risk of acquiring primary resistance to EGFR-TKIs when compared to patients with PD-L1 TPS < 50%, with an odds ratio (OR) of 16.47 (95% CI: 2.10–129.16, \( P = 0.008 \)) in a study of 66 surgically resected samples [177]. Gainor and colleagues [41] detected the level of PD-L1 in paired tumor tissues before EGFR-TKI treatment and tissues after development of resistance to EGFR-TKIs, and the results showed marked increases in PD-L1 expression in 12 patients (21%). Another study [176] reported that PD-L1 expression was increased in 7 patients (38.9%) with development of resistance to gefitinib, with high mesenchymal–epithelial transition (MET) activity (\( P = 0.028 \)). In vitro data also showed that PD-L1 expression is upregulated in cells resistant to gefitinib. Nevertheless, the underlying mechanism by which PD-L1 expression is associated with primary resistance to EGFR-TKIs remains unclear.

Future prospects
The application of EGFR-TKIs in combination with ICIs in routine clinical practice for patients carrying EGFR mutations has raised several concerns that have yet to be resolved by the multiple clinical trials conducted to date. Notably, traditional lung cancer treatments are far
simpler than the present situation demands. After failure of first-line TKIs, patients with EGFR mutations have limited treatment options [12, 179]. Thus, there is an urgent need to further investigate novel treatment strategies. This review summarizes some potential benefits in this regard. First, administering pembrolizumab prior to EGFR-TKI treatment is not recommended as the first-line treatment for EGFR-TKI-naïve, high PD-L1-expressing tumors, which is supported by a phase II trial (NCT0287994) [180]. The data showed that no EGFR-mutant NSCLC patients received a benefit from pembrolizumab administration prior to EGFR-TKI treatment [180]. Second, ICIs may be a promising approach for some cases with high PD-L1 expression or some uncommon EGFR-mutated NSCLC cases due to intratumor heterogeneity among PD-L1-expressing and EGFR-mutant clones [30, 118–120]. Third, tumor microenvironmental changes, a rather small window, may be most beneficial for combination EGFR blockade with immune-mediated anticancer approaches, which were found to be only temporary and disappeared as treatment continued [47]. Fourth, EGFR-TKIs may not be optimal for EGFR-TKI-naïve, high PD-L1 expressing, EGFR-mutated NSCLC as the first-line treatment due to a possible association between high PD-L1 expression and primary resistance to EGFR-TKIs [177, 178]. Fifth, the combination of VEGF/VEGFR inhibitors with ICIs may represent a new option for patients with EGFR mutations for whom TKIs have failed. Several possible mechanisms regarding immune-modulatory effects [181] through VEGF inhibition have been proposed, including T cell priming promotion and activation via DC maturation [181, 182], increased T cell tumor infiltration via normalization of the tumor vasculature through VEGF inhibition [183–185], and establishment of an immune-permissive TME via decreases in MDSC and Treg populations [181, 185]. However, the precise mechanisms of VEGF blockade and immune modulatory effects remain unclear. One randomized phase III trial (IMpower150) was conducted to test the efficacy of adding atezolizumab to standard-of-care bevacizumab and chemotherapy in NSCLC patients carrying EGFR mutations, and intriguingly, this approach has shown promising efficacy (median DOR: 11.1 months vs 5.6 months) compared with standard-of-care bevacizumab and chemotherapy [186]. Sixth, most EGFR-mutant advanced NSCLC patients harbor multiple co-occurring oncogenic mutations; thus, genomic molecular diagnosis should be applied to further select the most appropriate treatment strategy [187]. Given this complexity, it is essential to identify the optimal sequence of treatment and strategies for NSCLC patients with EGFR mutations (Fig. 3).

**Conclusion**

Globally, lung cancer has the highest rates of diagnosis and mortality among cancers. NSCLC accounts for more than 85% of all lung cancer cases, seriously threatening human health. Currently, immunotherapy is a very promising therapeutic strategy for NSCLC. Preclinical studies indicate that EGFR mutations mediate tumor

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**Fig. 3** Representative tumor characteristics and treatment regimens for NSCLC patients with EGFR mutations. TKI: tyrosine kinase inhibitors; EGFR: epidermal growth factor receptor; PD-L1: programmed death-ligand 1; ICIs: immune checkpoint inhibitors; T1: treatment 1; T2: treatment 2; Ate: atezolizumab; Bev: bevacizumab; Chemo: chemotherapy; (+): positive; (−): negative.
immune escape through the PD-1/PD-L1 pathway and that EGFR-TKIs downregulate PD-L1 expression [121, 125–130]. Overall, NSCLC Patients with EGFR mutations do not respond well to immunotherapy. However, some studies have shown that immunotherapy is still effective in patients with EGFR mutations. This review summarizes the current status of NSCLC patients with EGFR mutations who are treated with immunotherapy alone or in combination with EGFR-TKIs. During treatment with EGFR-TKIs, EGFR mutations may result in dynamic changes in the immunological profile, such as a dynamic immune TME, a low TMB, and altered expression of PD-L1. These contradictions and controversies suggest that immunotherapy or EGFR-TKI combination therapy in NSCLC patients with EGFR mutations requires steps of clinical validation and utility. Therefore, in this case, mechanisms to induce long-lasting antitumor activity in the TME and to maximize the effect of immunotherapy in patients can still be improved. There is also a clear unmet need for establishing prognostic molecular and clinical markers, dosages, schedules, the optimal sequence of treatment and strategies when combining immunotherapy with other therapies. We believe that the long-term survival of NSCLC patients with EGFR mutations may be very promising.

Abbreviations

(−): Negative; (+): Positive; ADO: Adenosine; Ate: Atezolizumab; ATEP: Adenosine triphosphate; Bev: Bevacizumab; CCL2: C-C motif chemokine ligand 2; CCR2: C-C motif chemokine receptor 2; CD8+ T cells: Cytotoxic T cells; Chem: Chemotherapy; CTIA: Class II major histocompatibility complex transactivator; CTLA-4: Cytotoxic T-lymphocyte–associated antigen 4; DCs: Dendritic cells; EGFR: Epidermal growth factor receptor; EGFR-TKIs: Epidermal growth factor tyrosine kinase inhibitors; Foxp3: Forkhead box P3; GSK-3β: Glycogen synthase kinase 3β; IFN-γ: Interferon-γ; IFN-γR: Interferon γ receptor; IL-10: Interleukin 10; Interleukin 10; MDSCs: Myeloid-derived suppressor cells; MEK/ERK: Extracellular signal-regulated kinase (ERK) kinase MEK-MHC: Major histocompatibility complex; M-MDSCs: Mononuclear MDSCs; Mcp: Macrophages; NKS: Natural killer cells; NSCLC: Non-small-cell lung cancer; ORR: Objective response rate; OS: Overall survival; PD-L1: Programmed death-ligand 1; PFS: Progression-free survival; PMN-MDSCs: Polymorphonuclear MDSCs; STAT3: Signal transducer and transcriptional activator-3; Tc: Tumor cells; Teffs: Effector T cells; TH1 cells: Type 1 helper cells; TIM-3: T cell immunoglobulin and mucin-domain containing-3; TME: Tumor microenvironment; Tregs: Treg cells

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