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Review

Research Progress in Immunotherapy of NSCLC With EGFR-Sensitive Mutations

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Lung cancer is a malignant tumor with high incidence and mortality across the world. The use of immune checkpoint inhibitors for lung cancer has improved the prognosis of some lung cancer patients to a greater extent and provided a new direction for the clinical treatment of lung cancer. Immunotherapy still has limitations in terms of its appropriate population and adverse reactions. Particularly for non-small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) mutation, there has been no major breakthrough in current immunotherapy. Whether immunotherapy can bring new benefits after drug resistance is induced by tyrosine kinase inhibitor-targeted therapy and whether the combination of immunotherapy with other treatments can improve the prognosis remain to be studied in depth. In this article, we provide a detailed review of the relevant characteristics of the tumor microenvironment of NSCLC with EGFR mutation and the current research on immunotherapy for NSCLC with EGFR mutation.

**Key words: Non-small cell lung cancer (NSCLC); Immunotherapy;
Epidermal growth factor receptor (EGFR)-sensitive mutations**

INTRODUCTION

According to the data of the Global Cancer Observatory (GLOBOCAN2020), the incidence and mortality of lung cancer in China account for 37.0% and 39.8% of those in the world, respectively. According to GLOBOCAN estimates, there were approximately 2.2 million new lung cancer cases worldwide in 2020, accounting for 11.4% of all malignant tumors, and there were approximately 1.8 million deaths, accounting for 18.0% of malignant tumor-related deaths¹. According to the data released by the National Cancer Center, 57 per 100,000 people in China had lung cancer in 2019, and approximately 630,000 people die of lung cancer each year². Lung cancer is also the malignant tumor with the heaviest burden in China³. From 2012 to 2015, the 5-year survival rate of lung cancer in the Chinese population was 19.7%, and most patients

were already at an advanced stage when they were diagnosed. With the combined application of chemotherapy, radiotherapy, targeted therapy, and immunotherapy, the prognosis of lung cancer has been improved, but in general, the 5-year survival rate has not been significantly improved⁴.

Non-small cell lung cancer (NSCLC) accounts for approximately 80%–85% of lung cancer, and the driver gene mostly determines the treatment and prognosis of NSCLC at present. The patients with NSCLC positive for the epidermal growth factor receptor (EGFR) gene have benefited significantly from the EGFR-tyrosine kinase inhibitor (TKI) targeted therapy. The marketed EGFR-TKIs have been continually optimized. The third-generation TKIs have shown a better therapeutic effect, and even the drug resistance problem of the T790M mutation of the first-generation TKIs has been better

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resolved. However, the discovery of various new mutation sites and the emergence of complex drug resistance targets also pose more challenges for targeted therapy. As research on immune checkpoint inhibitors (ICIs) progresses, can immunotherapy become a new treatment for NSCLC with EGFR-sensitive mutations? Programmed death-1 (PD-1) inhibitors may be less effective at treating NSCLC patients with EGFR mutations⁵⁻⁸. A meta-analysis including five trials (Checkmate 017, 057, Keynote 010, OAK, and POPLAR) showed that treatment with ICIs prolonged the overall survival (OS) in the EGFR wild-type (WT) subgroup [hazard ratio (HR) = 0.67, 95% confidence interval (CI) = 0.60–0.75; $p < 0.001$] but did not perform well in the EGFR-mutant subgroup (HR = 1.11, 95% CI = 0.80–1.53; $p = 0.540$)⁵. A similar analysis confirmed that ICIs did not increase the OS of NSCLC patients with EGFR mutations compared with patients taking docetaxel (HR = 1.09, 95% CI = 0.84–1.41)⁹. This has aroused the interest of many people, who have explored the treatment efficacy and studied the mechanisms of PD-1/programmed cell death ligand 1 (PD-L1) ICIs in patients with EGFR mutation. In this article, we will review their efforts in detail.

EFFECT OF EGFR MUTATIONS ON THE TUMOR IMMUNE MICROENVIRONMENT

In addition to cancer cells, primary tumors also include a variety of stromal cells, of which T lymphocytes, myeloid cells [myeloid-derived suppressor cells (MDSCs), mesenchymal stem cells (MSCs), mast cells, macrophages, and neutrophils], cytokines, and exosomes constitute the immunomodulatory network of tumors^{10,11}. The tumor microenvironment (TME) is an internal environment for the development and progression of tumor cells and promotes tumor metastasis. Interventions targeting the TME are an important part of tumor immunotherapy strategies¹⁰. The TME plays a specific role at different stages of tumor progression, gradually changing from the initial inhibition of tumor development and progression to a state of adaptation and promotion of tumor growth¹². T lymphocytes change from having an immune surveillance function to an immune escape function through immunoediting and even exhibit immunosuppression, such as by recruiting regulatory T cells (Tregs) and MDSCs and upregulating the levels of immunosuppressive cells in the TME. Tumor-associated macrophages (TAMs) induce tumor cell invasion by releasing cell colony-stimulating factor 1 (CSF-1) and macrophage-derived epidermal growth factor (EGF). Cancer-associated fibroblasts, inflammatory cells, and various growth factors and immunomodulatory cytokines are involved in tumor progression⁷⁻⁹.

NSCLC with EGFR mutations has a unique TME that is mainly in a noninflammatory state. In this state, the

inflammatory response around the tumor cells is weak, lymphocyte infiltration is reduced, and antitumor immunity is lowered. EGFR mutations can regulate potential factors associated with TME status through multiple pathways, such as through tumor-infiltrating lymphocytes (TILs)¹³⁻¹⁵, Tregs^{16,17}, MDSCs^{18,19}, TAMs, and immunomodulatory cytokines²⁰.

EGFR Mutation and Tregs

Tregs are physiologically involved in maintaining immune tolerance and immune homeostasis. Under pathological conditions, Treg cells are found at a high frequency in various types of cancer and are considered to be a key obstacle to antitumor immunity. Forkhead box P3 (Foxp3) is one of the key transcription factors controlling the development and function of Tregs. Induction of Foxp3 expression in naive T cells in vivo or in vitro can lead to Treg-like immunosuppressive effects²¹⁻²³. Cytokines such as transforming growth factor- β , interleukin (IL)-10, and IL-35 secreted by Treg cells can create an immunosuppressive environment that actively attenuates and inhibits the antitumor immune response of CD4⁺ T cells, CD8⁺ T cells, and natural killer cells^{9,24,25}. On the other hand, Tregs constitutively express inhibitory molecules such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), PD-1, and TIM-3, which bind to specific ligands to facilitate their inhibitory function.

The expression of indoleamine 2,3-dioxygenase (IDO) can upregulate Treg function and induce immune tolerance^{26,27}. Huang et al. found that EGFR-containing exosomes can induce plastic transformation of tolerogenic dendritic cells (DCs) and cause DCs to produce IDO, which plays an important role in converting CD4⁺CD25⁻ T cells into CD4⁺CD25⁺ Treg cells²⁸. Amphiregulin (AREG) is one of the ligands of EGFR, and its higher expression in the plasma of NSCLC patients is associated with a poor prognosis²⁹. In addition, as a specific molecule in the exosomes of tumor cells, amphiregulin plays an important role in promoting tumor progression³⁰. Wang et al. found that amphiregulin can regulate the suppressive function of Treg through the EGFR/GSK-3/Foxp3 axis both in vitro and in vivo³¹. The EGFR-TKI gefitinib inhibits EGFR activity, restores GSK-3 β activity, and attenuates Treg function³². Mascia et al. also showed that knockdown of EGFR expression significantly inhibited tumor cell growth and downregulated Treg infiltration into the TME³³.

EGFR Mutation and TILs

TILs are a group of tumor-infiltrating and antigenic cell populations that are present in tumor nests and stroma³⁴. CD8⁺ T cells act as antitumor immune cells in the TME to destroy malignant tumor cells by releasing cytokines such as interferon- γ , perforin, and granzyme B,

and the number of CD8⁺ T cells determines the tumor cell killing rate. A high degree of infiltration of CD8⁺ TILs in NSCLC is associated with a good prognosis and treatment outcome^{35–38}.

Teng et al. evaluated the efficacy of immunotherapy by establishing a TME model based on TIL and PD-L1 expression, suggesting that an immune-inflammatory TME (PD-L1⁺ and TIL⁺) is most likely to benefit from anti-PD-1/PDL1 therapy³⁹. Some patients with EGFR mutations have lower TIL levels as well as a lower ratio of PD-L1⁺CD8⁺ TILs^{14,15,40}. The decrease in CD8⁺ TIL levels may be related to the low expression of major histocompatibility complex (MHC) molecules. The MHC plays an important role in tumor antigen presentation. The tumor antigens of MHC class I molecules constitute the first signal for cell activation, they activate CD8⁺ T cells, and they play a role in the antitumor immune effect⁴¹.

EGFR Mutation With TAM and Immunoregulatory Suppressors

Macrophages are an important component in the TME, and it is well established that macrophages can polarize into two states in response to different microenvironmental stimuli, namely, classically activated M1 macrophages and alternatively activated M2 macrophages. The former typically show inflammatory functions, whereas the latter show anti-inflammatory functions. TAMs are thought to more closely resemble M2-polarized macrophages and facilitate tumor growth by inducing immune suppression. For NSCLC with EGFR mutations, whose TME is in a noninflammatory state, Chen et al. showed that EGFR activation can recruit M2 macrophages to suppress antitumor immunity by inducing the expression of ILT4⁴². Wang et al. analyzed data among 515 human lung adenocarcinomas from the TCGA database and found that enumeration of alveolar macrophages (AMS) by CIBERSORT revealed a higher number of AMS in 32 EGFR-mutant tumors compared with 408 EGFR WT/KRAS WT tumors ($p = 0.036$) and 75 KRAS-mutant tumors. Their study showed that AMS promoted tumor progression in EGFR mutated NSCLC ($p = 0.073$)⁴³. In addition, activation of the EGFR pathway can induce the release of multiple immunosuppressive factors, including TNF- β [transforming growth factor- β (TGF- β)], IL-10, vascular endothelial growth factor (VEGF), IDO, CC chemokine ligand 2 (CCL-2), arginase (ARG)-1 and adenosine, etc.⁴⁴. These immunosuppressive agents can directly inhibit the killing of natural killer cells, the maturation of DCs, and the proliferation and function of cytotoxic T cells. Moreover, it can further inhibit the host antitumor immunity by promoting the transformation of CD3⁺CD4⁺CD25⁻ cells to CD4⁺CD25⁺FoxP3⁺ Tregs, inducing the polarization of TAM into M2 macrophages, and mediating the recruitment and activation of MDSCs⁴⁵.

EGFR Mutation and MDSCs

MDSC is an immature and dysfunctional myeloid cell group differentiated from immature bone marrow cells (IMCS) under a variety of pathological conditions such as inflammation, infection, and cancer. It has a strong immunosuppressive function. MDSC, Treg, and Tam together constitute the main immunosuppressive cells of TME in patients with lung cancer. It has been reported that MDSC in lung cancer can be divided into two types, namely, monocyte CD33⁺CD11b⁺CD14⁺ MDSCs or granulocyte-like CD33⁺CD11b⁺CD14⁻ MDSCs⁴⁶. In EGFR mutant NSCLC, the activation of EGFR pathway can produce some cytokines (such as IDO and VEGF). These cytokines can induce IMCs to differentiate into MDSC and recruit MDSCs into TME, so as to play the function of immunosuppression. Continuous activation of EGFR signaling pathway in tumor cells can induce the activation of downstream STAT3, which is the main transcription factor regulating MDSC amplification⁴⁷. However, there is no clear answer to whether EGFR mutation exists in IMC. Generally speaking, the activation of EGFR signaling pathway can lead to the activation of downstream signaling networks that affect multiple processes of cells, which may be the reason why EGFR-mutant NSCLC shows a TME significantly different from EGFR WT lung cancer, and MDSCs are an important participant in the formation of this noninflammatory microenvironment.

EGFR MUTATIONS CAUSE REDUCED IMMUNOGENICITY IN TUMOR CELLS

EGFR Causes Changes in Tumor Mutation Burden (TMB)

TMB is the total number of substitution, insertion, and deletion mutations per megabase in the coding region of tumor genes. It is a good biomarker for predicting the efficacy of immunotherapy. It can quantitatively estimate the total number of mutations in the coding region of tumor genomes. Higher TMB is associated with more neoantigens produced by tumors, easier recognition by immune cells, and long-term clinical responses⁴⁸.

Cancer cells can produce tumor-specific antigens and tumor-associated antigens, which are processed by antigen presentation machinery to activate effector lymphocytes against them⁴⁹. Tumor-specific antigens are defined as neoantigens present only on the surface of tumor cells but not on the surface of normal cells that mainly specifically induce the activation of T lymphocytes to produce CTLs and exert antitumor effects. Neoantigens can be generated by mutations of oncogenes or tumor suppressor genes, chromosomal translocations, and viral genes encoding new peptides. When tumors show a greater mutation load, T-cell differentiation may be better

stimulated, and T-cell clonal expansion will produce a larger antitumor immune cell bank⁵⁰. We can measure the value of TMB by the total number of nonsynonymous mutations in each coding region of the tumor genome. The nonsynonymous mutation number is more closely related to TMB than the total exon mutation burden⁵¹. For the treatment of NSCLC with ICIs, a higher TMB enables a faster response and better clinical efficacy^{52,53}. In practice, however, the survival rate of patients with high TMB may be worse without ICI treatment⁵².

Patients with EGFR mutations show lower neoantigen burden and fewer nonsynonymous mutations⁵⁴, and the latter are negatively correlated with the clonality of T-cell receptor β ⁵⁵. Patients with EGFR mutations have significantly lower TMB than those with WT EGFR, which may be related to the smoking history of EGFR-mutant NSCLC. The number of point mutations in lung cancer patients who smoke is 10 times that of never-smokers, which shows that smoking history is significantly associated with the increase in TMB⁵⁶. A meta-analysis showed that the chance of having EGFR mutations in never-smokers is significantly higher, while the number of patients with EGFR mutations decreases as the smoking history increases⁵⁷. Recently, the possibility that traditional chemotherapy can alter TMB has been explored. Studies on EGFR mutation sites have found that TMB may also differ between mutation sites, and tumors with the EGFR L858R mutation have been confirmed to have higher TMB and better response to ICIs⁵⁸.

EGFR Mutations Cause Changes in the Antigen Presentation Machinery

Mitogen-activated protein kinase (MAPK) signaling inhibits the antigen presentation machinery, while the inhibition of MAPK signaling broadly upregulates it. Brea et al. activated the MAPK pathway by inducing EGFR mutations and found that in addition to inducing the upregulation of immune checkpoint blockade, it caused the inhibition of MHC-I in vivo. EGFR mutations led to the downregulation of the expression of MHC class I molecules, resulting in weak antigen-presenting ability and low immunogenicity⁵⁹.

The expression of MHC-I and MHC-II can be achieved through the interferon- γ (IFN- γ) signal transduction pathway and the downstream MEK/ERK signal transduction pathway⁶⁰⁻⁶³. Watanabe et al. found that the combination of an EGFR-TKI (irreversible EGFR-TKI afatinib or trametinib) and IFN- γ had an additive effect on HLA expression and the expression of antigen peptide transporter TAP1 and β 2-microglobulin⁶⁴. We can deduce that MHC molecules are expressed more highly when the EGFR pathway is inhibited, further confirming that EGFR mutations are negatively correlated with the expression of MHC molecules.

EGFR MUTATION AND PD-L1 EXPRESSION

Mechanism of PD-L1 Expression

PD-L1 is one of the ligands of T-cell immune checkpoint PD-1 that is widely expressed in hematopoietic and parenchymal cells. It participates in T-cell autoimmune tolerance and prevents itself from being attacked by the immune system under chronic inflammation or infection. In a variety of solid tumors, PD-L1 is overexpressed to protect tumor cells from being damaged by cytotoxic T cells, thereby forming an immunosuppressive TME and promoting tumor progression^{65,66}. The expression mechanisms of PD-L1 in tumor cells can be divided into constitutive oncogene activation expression and cytokine-induced expression, and oncogene activation expression is constitutive and diffuse⁶⁷. In a variety of tumors, the loss of phosphatase and tensin homolog can constitutively upregulate the expression of PD-L1 on the surface of tumor cells, and dysregulation of the Janus-activated kinase/signal transducer and activator of transcription (STAT) pathway can also lead to oncogene-driven PD-L1 overexpression. Coelho et al. found that KRAS activating mutations can enhance the stability of PD-L1 mRNA through the MEK-ERK signaling pathway⁶⁸, and multiple different signaling pathways can participate in the regulation of PD-L1. The inflammatory status of the tumor TME plays an important role in the upregulation of PD-L1 expression in the process of cytokine-induced upregulation of PD-L1. In the inflammatory microenvironment, T cells mount a classical inflammatory response and secrete a variety of cytokines to induce PD-L1 expression on tumor cells. Cytokines including IL-1 α , IL-6, TNF- α , and IFN- γ promote PD-L1 expression on tumor cells in a dose-dependent and additive manner. Among them, IFN- γ is the main stimulating factor of PD-L1⁶⁹.

Effect of EGFR Mutation on PD-L1 Expression

The EGFR signaling pathway is one of the most important oncogenic pathways in NSCLC⁷⁰. The activation of EGFR signal transduction not only impacts tumor biology but also can regulate host antitumor immunity, including by driving PD-L1 expression and impeding TIL activation⁷¹. The expression level of PD-L1 in NSCLC cell lines with mutated EGFR was found to be significantly higher than that in cells with WT EGFR⁷². After the activity of EGFR was blocked by EGFR-TKIs, NSCLC cell lines with EGFR mutations downregulated their PD-L1 expression. The transfection of BEAS2B immortalized bronchial epithelial cells with mutated EGFR resulted in increased PD-L1 expression at the mRNA and protein levels. In mouse models, EGFR mutations activated PD-L1 expression and induced immune escape, whereas treatment with EGFR-TKIs downregulated PD-L1 expression⁷³.

From these results, we can infer that the transduction of oncogenic EGFR signaling can directly or indirectly drive PD-L1 upregulation. The PI3K–Akt–STAT3/mTOR signaling pathway is involved in EGFR-mediated PD-L1 expression⁷⁴. The activation of mTOR upregulates the expression of PD-L1 by inducing its translation rather than transcription. IFN- γ -mediated PD-L1 expression also depends on mTOR. Inhibition of Akt or STAT3 activity may downregulate PD-L1 expression even in gefitinib-resistant NSCLC⁷⁵. There is also evidence that EGFR activation can upregulate PD-L1 through the ERK1/2 pathway⁷⁶. Contrary to what was expected, patients with mEGFR have tended to have worse remission than those with WT EGFR during anti-PD-1/PD-L1 clinical treatment. The reason for the poor therapeutic effect may be that the upregulation of EGFR-mediated PD-L1 expression induced by the PI3K–Akt–STAT3/mTOR signaling pathway and the mEGFR-induced decrease in IFN- γ occur simultaneously, so it is difficult to estimate whether PD-L1 expression is increased in reality^{69,70}.

This relationship is paradoxical in the clinical analysis of NSCLC patients with EGFR mutations. Tang et al. analyzed the tissues of 170 patients with lung adenocarcinoma by immunohistochemical assays, and the results showed that PD-L1 overexpression may be related to EGFR mutations⁷⁷. However, two pooled analyses^{78,79} confirmed that PD-L1 expression was negatively correlated with EGFR mutation. The researchers evaluated the tissue sample data of 3,969 patients by analyzing the immunohistochemical assays of 18 studies and found that EGFR-mutant NSCLC was less likely to be PD-L1 positive than WT EGFR tumors [odds ratio (OR) = 0.59, 95% CI = 0.39–0.92; $p < 0.02$]⁸⁰. Liu et al. found that EGFR-WT NSCLC tumors were more likely to be PD-L1 positive than EGFR-mutant tumors (OR = 1.79, 95% CI = 1.10–2.93; $p = 0.02$)⁸¹. In addition, they analyzed the mRNA and protein levels of PD-L1 in the Cancer Genome Atlas and internal database (Guangdong Lung Cancer Institute) and detected lower PD-L1 mRNA and PD-L1 protein expression in EGFR-mutant NSCLC samples than WT tumor samples. These opposing results may be related to the differences in how PD-L1 expression was measured (i.e., immunohistochemical analysis vs. mRNA expression). For now, it remains controversial how EGFR mutations influence PD-L1 expression.

EXPLORATION ON THE COMBINED APPLICATION OF ICIs AND EGFR-TKIs

Effects of EGFR-TKIs on the TME

TKIs can increase IFN secretion to enhance the induction of MHC-I and MHC-II molecules, promote Foxp3 degradation to attenuate the suppressive function of Tregs⁸¹, enhance antigen-specific antitumor T-cell

responses, reduce T-cell apoptosis, and increase the cytotoxicity of CTLs that can enhance the immune system responses⁷⁹. Therefore, the combined application of EGFR-TKIs may show some effect in anti-PD-1/PD-L1 treatment.

Effect of Treatment With EGFR-TKIs on PD-L1 Expression

The expression of PD-L1 may be dynamic and may be inhibited during EGFR-TKI treatment, but some patients show increased PD-L1 expression after displaying acquired resistance to EGFR-TKIs. Gainor et al. examined the levels of PD-L1 in paired tumor tissues that were taken either before EGFR-TKI treatment or after the development of resistance to EGFR-TKI, and the results showed that PD-L1 expression was significantly increased in 12 patients (21%)¹³. The researchers also found that the frequency of PD-L1 expression in patients with EGFR mutations was lower before TKI exposure (16%; PD-L1 $\geq 5\%$) than after acquired resistance to TKI had occurred (29%; PD-L1 $\geq 5\%$). Therefore, the authors concluded that the development of primary resistance to EGFR-TKIs in NSCLC patients with EGFR mutations seems to be related to the upregulation of PD-L1 expression on the tumor surface.

Combined Application of TKIs and Immunosuppressive Agents

Some clinical trials have tested the combinations of PD-1 or PD-L1 inhibitors with EGFR TKIs, targeting TKI-pretreated or untreated patients with EGFR mutations. In a phase I clinical trial, the investigators treated 20 NSCLC patients with EGFR mutations who had received TKI therapy with erlotinib in combination with nivolumab, and the results showed an objective response rate of 15% (3/20), but five patients experienced treatment-related grade 3 toxicity events in this study⁸². TATTON was a phase Ib trial that combined osimertinib (third-generation TKI) with selumetinib (MEK1/2 inhibitor), savolitinib (MET-TKI), or durvalumab (anti-PD-L1 monoclonal antibody) for the treatment of EGFR-mutant NSCLC. Because of the increased incidence of interstitial lung disease in the durvalumab group, this arm of the trial was terminated⁸³.

CAURAL is a phase III randomized trial that was also terminated early due to safety concerns given the high incidence of interstitial lung disease reported in the TATTON trial. In a phase Ib trial, erlotinib was administered in combination with atezolizumab, and the initial results showed an objective response rate of 75% (95% CI = 50.9–91.3) and a median duration of response (DOR) of 16.7 months (range = 4.2–26.0). Although no grade ≥ 4 adverse events occurred, grade 3 treatment-related adverse events were reported in 43% of patients⁸⁴. Indeed, toxicity is the main

concern of the combination strategy. In the trial on gefitinib combined with durvalumab, grade 3 and grade 4 hypertransaminasemia occurred in 40% and 15% of patients, respectively, and 20% of patients had to discontinue treatment because of treatment-related adverse events⁸⁵. Clinical trials combining TKIs and ICIs are still ongoing, and most of them still need to assess safety and tolerability. The reason behind the increased toxicity remains unclear, but current studies show that these combinations have no significant advantage in therapeutic efficacy, but they show more severe toxicity and side effects.

EGFR MUTATIONS AND TREATMENT WITH ICIs

We found that patients with EGFR mutations did not respond well to immunotherapy in the Checkmate 017, 057, Keynote 010, OAK, and POPLAR studies, which then raises questions as to whether there are differences between different mutation sites? It is known that EGFR mutations occur at several hotspots between exons 18 and 21. The in-frame deletion in exon 19 (EGFR Δ 19) and the L858R point mutation in exon 21 were the most common mutations detected, accounting for 50% and 40% of patient samples, respectively⁸⁶. Both types of mutations are sensitizing mutations, and tumors with these mutations are sensitive to EGFR TKIs. A special type of EGFR mutation, an in-frame insertion in exon 20, occurs in 3%–7% of NSCLCs, and most tumors with this type of mutation are resistant to treatment with EGFR TKIs⁸⁷. Hastings et al. treated 126 patients with the most common EGFR gene mutations (EGFR Δ 19 and EGFR L858R) with ICIs and compared them with 212 patients with WT EGFR⁸⁸. The researchers found that patients with EGFR Δ 19 had a lower objective response rate than those in the WT EGFR group ($p = 0.002$), whereas there was no difference between the WT EGFR group and the EGFR L858R group. In terms of OS benefit, there was no difference between WT EGFR and EGFR L858R, whereas shorter OS was found in the EGFR Δ 19 group ($p = 0.069$ and $p = 0.03$, respectively). Haratani et al. assessed the response of the EGFR resistance mutation T790M to the treatment with ICIs⁸⁹. The results suggested that treatment with ICIs may provide poorer survival outcomes in this subgroup of patients than in those with nonresistant mutations. The authors suggested that ICIs may achieve certain results in patients whose condition progressed after first-line TKI treatment before they developed the secondary T790M mutation. Yamada et al. retrospectively analyzed 27 patients with advanced NSCLC with EGFR mutations (rare or T790M) whose condition progressed after TKI treatment⁹⁰. They found that the efficacy and best outcome of immunotherapy were correlated with having a rare EGFR mutation or not having the T790M mutation. This result suggested the possible use of ICIs,

such as the later treatment for patients with rare EGFR mutations but not the T790M mutation whose condition progressed after TKI treatment. In addition, data from the ImmunoTarget registry presented at the American Society of Clinical Oncology (ASCO) 2018 meeting showed that NSCLC patients with the T790M mutation or EGFR Δ 19 mutation who were treated with ICIs had worse survival than those having other types of mutations⁹¹.

In summary, further analysis of the different EGFR mutations is necessary for improved selection of patients with EGFR mutations who may be more responsive to ICIs. At present, it remains unclear as to the therapeutic efficacy of ICIs in NSCLC patients with EGFR mutations whose conditions have progressed after TKI treatment. The key question remains whether ICIs have a definite benefit in NSCLC patients with EGFR mutation. For EGFR-mutant NSCLC, factors leading to weak immunogenicity, such as TME not yet in an inflammatory state, lack of PD-L1⁺/CD8⁺ TILs, and low TMB^{92,93}, will limit the antitumor immune response, even in a subgroup of patients with higher PD-L1 expression. Therefore, the clinical application of ICIs in patients with EGFR-mutant NSCLC still needs further study.

ANTIANGIOGENIC DRUGS MAY IMPROVE THE IMMUNE RESPONSE IN PATIENTS WITH EGFR MUTATIONS

Antiangiogenic drugs can directly or indirectly improve the immune microenvironment of tumors. In fact, in the process of tumor progression, the abnormal vascular proliferation around the tumor inhibits the body's antitumor immunity roughly through the following three aspects: 1) the lack of pericyte coverage, the widening of the endothelial cell gap, and the breakage or loss of the basement membrane of the new blood vessels increase the leakage of blood vessels, resulting in increased interstitial fluid pressure, which hinders the infiltration of T cells; 2) the nascent vasculature often lacks some adhesion molecules, which further inhibits the response of immune cells; and 3) tumor blood vessels are morphologically characterized by tortuosity, distension, high disorder, and uneven thickness, which lead to blood flow disturbances and hypoxia⁹⁴. The hypoxic environment can help to upregulate some signals that inhibit antitumor immune responses, such as PD-L1, IDO, IL-6, and IL-10, and the hypoxic TME can also promote the polarization of TAMs into an M2-like phenotype⁹⁵. Therefore, antitumor angiogenic drugs can induce the normalization of tumor blood vessels and induce TAM differentiation into M1-like phenotype by alleviating hypoxia⁹⁶. There is increasing evidence that appropriate administration of antiangiogenic drugs can induce changes in the state of the TME, that is, from an immunosuppressive state to an improved antitumor immunity state⁹⁷. Anti-VEGF drugs can also block circulating VEGF-mediated

blocking effects that hinder the maturation and differentiation of DCs⁹⁸. The combined application of ICIs and anti-angiogenic drugs has also been studied in clinical trials. NCT00790010 investigated the role of ipilimumab (anti-CTLA-4) and bevacizumab (anti-VEGF) in patients with metastatic melanoma, and the results showed that patients receiving the combined treatment had a better prognosis⁹⁹. Wallin et al. treated 10 patients with previously untreated metastatic renal cell carcinoma with the combination of atezolizumab (anti-PD-L1) and bevacizumab, and eight of them showed a favorable response¹⁰⁰. The Impower150 study is a multicenter, open-label, randomized, phase III study that evaluated the efficacy and safety of atezolizumab plus chemotherapy with or without bevacizumab versus bevacizumab in combination with chemotherapy in patients with nonsquamous NSCLC. The results of IMpower150 showed that immunotherapy combined with antiangiogenic agents and chemotherapy yielded significant benefits in both PFS and OS in first-line treatment of nonsquamous NSCLC. The study was divided into three groups, the treatment regimen of group A was atezolizumab + paclitaxel + carboplatin (ACP), the treatment regimen of group B was atezolizumab + bevacizumab + paclitaxel + carboplatin (ABCP), and the treatment regimen of group C was bevacizumab + paclitaxel + carboplatin (BCP)¹⁰¹. A subgroup analysis of patients with EGFR-sensitive mutations in the Impower150 trial showed that the ABCP regimen also provided significant OS benefits. Median OS was improved by 11.3 months, 29.4 versus 18.1 months, HR = 0.6 (95% CI = 0.31, 1.14), in ABCP versus BCP. In addition, no OS benefit was observed in the ACP group compared with the BCP group. The incidence of treatment-related grade 3–4 adverse events was 21 (64%) of 33

patients in the ABCP group, 30 (68%) of 44 patients in the ACP group, and 28 (64%) of 44 patients in the BCP group. No patients in the ABCP and ACP groups experienced grade 5 adverse events. One patient in the BCP group experienced a grade 5 adverse event¹⁰². In the safety analysis of Impower150, when compared with the three-drug ACP and BCP regimens, the four-drug ABCP regimen has no additional toxicity and are relatively safe and tolerable. The findings of Impower150 provide a new direction and possibility for immunotherapy. It is certain that the normalization of tumor blood vessels has a great promoting effect on antitumor immunity, but whether the combination of ICIs and antiangiogenic drugs can become a new possibility for the treatment of EGFR-mutant NSCLC needs further study¹⁰³. Please see Figure 1 for a graphical summary of this review.

OUTLOOK

There is increasing evidence that ICIs provide clinical benefit to cancer patients, and immunotherapy will play an increasingly important role in cancer therapy in the future. For EGFR-mutant NSCLC, it is important to determine whether patients can benefit from ICI treatment. Different types of EGFR mutations may exhibit different responses to ICIs, and it is necessary to explore the mechanism by which patients with EGFR mutations benefit from ICI treatment. Understanding how to improve the immunogenicity of EGFR-mutant NSCLC will provide much-needed evidence for the application of immunosuppressive agents. Therefore, many clinical trials and basic studies are still needed to explore the mechanism, potential influencing factors, and biomarkers of the response to ICIs. It is believed that future studies can clarify or improve the immunotherapy for EGFR-mutant NSCLC and can continue to extend the good prognosis of patients with EGFR-mutant NSCLC.

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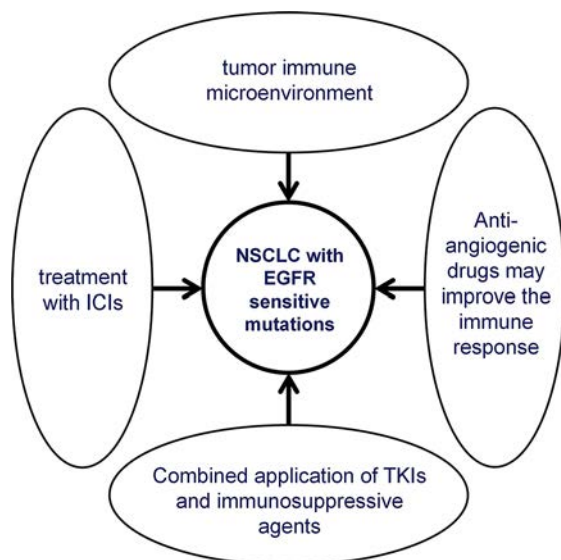


Figure 1. Graphical summary for the text.

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