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The Resistance Mechanism of ICI to EGFR and the Improvement Strategies

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Abstract:

Lung cancer is the leading cause of cancer-related deaths worldwide, among which non-small cell lung cancer (NSCLC) is the most common pathological subtype. Immune checkpoint inhibitors (ICIs) significantly improve the survival prognosis of patients with advanced lung cancer and enhance their quality of life by targeting molecules such as PD-1/PD-L1. However, the efficacy of ICI varies significantly. Due to the presence of EGFR mutant patients (over 50%) in the NSCLC population, these patients are insensitive to PD-L1 ICI immunosuppressive agents, resulting in limitations of PD-L1 ICI therapy. Therefore, it is necessary to systematically review the current application status, resistance mechanisms, and biomarker optimization strategies of ICI in EGFR mutant patients. This article summarizes the efficacy differences of ICI in different EGFR mutant subtypes, discusses the resistance mechanisms of ICI, and points out the optimization direction of ICI for the treatment of EGFR mutant-type lung cancer. Finally, it is found that although PD-L1 is highly expressed in EGFR mutant patients, they still have resistance; the factors of resistance include intrinsic factors and microenvironment factors; neoadjuvant immunotherapy (such as IMpower130) provides prospects for ICI treatment, but the biomarkers need to be further optimized. In conclusion, ICI has provided breakthrough progress in the treatment of EGFR-type lung cancer, but it still needs to solve the resistance problems and limitations in treatment. This article, through reviewing the above content, provides a reference for the precise decision-making and future research directions of EGFR mutant-type lung cancer treatment.

Keywords:- Lung cancer; immune checkpoint inhibitors (ICI); EGFR mutant type; resistance mechanism; therapeutic efficacy difference

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I. Introduction *(Heading 1)*

Lung cancer is the leading cause of cancer-related deaths worldwide, of which non-small cell lung cancer (NSCLC) is the most common pathological subtype. NSCLC is one of the malignant tumors with the highest morbidity and mortality in the world, and its pathological type accounts for about 80% of all lung cancers. Conventional treatments such as surgical therapy, radiotherapy, and platinum-based chemotherapy plans can extend the life span of patients to a certain extent, but the effectiveness is relatively low and may cause significant side effects. With the in-depth study of the mechanism of tumor immune escape and the development of immune checkpoint inhibitors (ICI) such as programmed death ligand 1 (PD-L1) and its ligand PD-L1, immunotherapy has gradually become a new hope for the treatment of advanced NSCLC. ICI can relieve immunosuppressive signals to restore the activity of T cells. Existing PD-1/PD-L1 inhibitors can relieve the immunosuppressive state of T cells, thereby restore their tumor-killing function and occupy a dominant position in the field of lung cancer ICI. With the continuous deepening of the study of immune escape mechanisms, ICI therapy has gradually become an important treatment option for patients with advanced NSCLC, especially in patients with high expression of PD-L1, the efficacy of its monotherapy has been significantly improved. Although ICI has shown some efficacy in NSCLC, the efficacy of existing ICI treatments still has significant limitations. For patients with epidermal growth factor receptor (EGFR) mutations, ICI resistance is a prominent problem, and there is an urgent need to explore combined treatment strategies to overcome resistance mechanisms. EGFR mutant NSCLC patients account for approximately 50% of all NSCLC patients, and these patients generally have resistance to ICI immunotherapy targeting PD-L1 [1]. EGFR mutations lead to resistance to anti-PD-L1 ICIs, which may be related to the infiltration of immunosuppressive cells (such as regulatory T cells, Tregs) and T cell exhaustion in the tumor microenvironment (TME) [2].

Current studies have found that the drug resistance mechanism of EGFR mutant tumor cells involves complex regulation of tumor cells, TME and host system [3]. TME plays a key role in tumor occurrence, development and drug resistance. TME is composed of tumor cells, stromal cells, immune cells, vascular endothelial cells, and a variety of bioactive factors and signal transduction molecules, forming a complex and dynamic ecosystem. Among them, immune escape, as one of the typical characteristics of malignant tumors, is closely related to a variety of immunosuppressive mechanisms in TME, such as abnormal activation of immune checkpoint receptors and their ligands, thereby inhibiting the cytotoxic activity of T cells.

These factors interact with each other, helping tumor cells evade immune surveillance and clearance through the "3C" theory of camouflage, coercion, and cytoprotection, thus affecting the efficacy of immunotherapy. Among them, T cell exhaustion and Treg-mediated immunosuppression are key barriers to the efficacy of ICI.

To overcome these problems, a number of current clinical studies are exploring combined treatment strategies including anti-angiogenic drugs and epigenetic regulators to enhance the efficacy of ICI and reverse the immune tolerance state [4]. For patients with EGFR mutations, neoadjuvant immunotherapy (such as the IMpower030 study) combined with existing ICI therapy has been shown to show a high pathological complete remission rate in patients with operable NSCLC, providing them with potential cure opportunities.

This article reviews the latest clinical research progress, refers to domestic and foreign literature, summarizes the differences in the efficacy of ICI in EGFR mutations, explores the resistance mechanism of EGFR, and points out the application prospects of ICI in the treatment of EGFR mutations and the optimization direction of markers.

II. 1. Background of ICI treatment for EGFR mutations in NSCLC

A. Efficacy Advantages for People with High PD-L1 Expression

NSCLC is the malignant tumor with the highest morbidity and mortality in the world, and its pathological type accounts for about 85% of all lung cancers. With the update and iteration of medical technology, ICI has been widely used in the field of lung cancer and can improve patient survival. In existing clinical treatments, the common treatment method of ICI is to enhance tumor-specific T cell immunity by inhibiting PD-1/PD-L1 signaling, which has shown good results in the treatment of NSCLC. Leora Horn from Vanderbilt University Medical Center (L. Horn) found that atezolizumab, a representative of PD-L1 inhibitors, achieved significant efficacy in the treatment of advanced NSCLC. Leora Horn randomly assigned patients in a 1:1 ratio to receive carboplatin and etoposide combined with atezolizumab or placebo for 4 21-day cycles, followed by a maintenance period during which patients received atezolizumab or placebo (according to the previous random assignment scheme), and then evaluated the treatment effects of the two groups. The results showed that the median overall survival (mOS) of the atezolizumab group and the placebo group were 12.3 months and

10.3 months, respectively, and the overall survival of the atezolizumab group was significantly longer than that of the placebo group. Although it has obvious curative effect, the emergence of drug resistance is still unavoidable, and studies have found that pembrolizumab therapy may cause related liver damage. Due to the difficulty in detecting adverse drug reactions, it is necessary to add anticoagulants after excluding pathological drug reactions, and strengthen the postoperative and prognosis monitoring of patients.
ICI treatment shows anti-tumor activity and controllable safety, but its overall effectiveness still needs to be strengthened. Therefore, traditional chemotherapy is still a common treatment for EGFR mutants that develop resistance. However, the traditional dual-drug combined chemotherapy regimen as a second-line treatment after EGFR-TKIs resistance still cannot avoid the continuous progression of patient tumors, and the treatment effect is limited. From the perspective of existing ICI and its application direction, the treatment range of ICI has gradually expanded from advanced lung cancer to early lung cancer. Innovative strategies are needed to improve the efficiency of ICI treatment, which can provide potential cure opportunities for surgical patients.

B. Differences and Mechanisms of ICI Response in Patients with EGFR Mutations

Although ICI treatment has shown high anti-tumor activity and high safety, there are still some patients who cannot benefit from immunotherapy (primary resistance), and some patients relapse after a period of immune response (acquired resistance). The reasons for the differences in the response of EGFR mutant NSCLC to ICIs are mainly due to the intrinsic factors of tumor cells and TME factors.
EGFR is a receptor tyrosine kinase. When EGFR mutates, it can lead to abnormal activation of signaling pathways, promote tumor cell proliferation and metastasis, and cause tumor cells to become resistant to ICI. The reasons for the differences in the response of EGFR mutant NSCLC to ICI are mainly due to the intrinsic factors of tumor cells and TME factors. Tumor cell intrinsic factors that lead to resistance to immunotherapy include the expression or inhibition of certain genes and pathways in tumor cells, which prevent immune cells from infiltrating or playing a role in TME. In addition to the intrinsic factors of tumor cells, the exogenous factors that cause tumor cell resistance include Tregs, myeloid-derived suppressor cells (MDSCs), M2 macrophages, and other inhibitory immune checkpoints, which may inhibit the effect of ICI in the treatment of NCSLC. Among them, epidermal growth factor receptor (EGFR) plays a major role in NSCLC tumor cells [5].
1) Tumor intrinsic factors
The intrinsic resistance mechanism of tumors to ICI mainly involves three aspects: antigen processing and presentation dysfunction, IFN-γ signaling pathway abnormalities, and inherited T cell rejection. These mechanisms together constitute a multi-level defense system for tumors to escape immune surveillance.
EGFR mutations can affect the antigen presentation mechanism. Some studies have found that the expression of MHC-I molecules in EGFR mutant tumors is downregulated, resulting in the inability of tumor-specific antigens to be effectively processed and presented to the cell surface, thereby hindering T cells from recognizing and killing tumors. The functional loss of β2-microglobulin (B2M) or the downregulation of HLA-I molecule expression can lead to complete MHC-I molecule assembly disorders, allowing tumor cells to gain immune escape capabilities.
The interferon-γ (IFN-γ) pathway is one of the key factors in the development of resistance to ICI inhibitor therapy. As a key cytokine secreted by activated T cells and NK cells, it plays multiple regulatory roles in anti-tumor immune responses. IFN-γ participates in immune surveillance by inducing tumor cell apoptosis, upregulating the expression of MHC-I class molecules, and enhancing the efficiency of antigen presentation. Active IFN-γ signaling is a predictive marker for PD-1 blockade response. Tumor cells can produce IFN-γ resistance through a variety of mechanisms, including JAK1/2 gene mutations and upregulation of STAT1/3 signal inhibitors. Mutations or epigenetic silencing of molecules in the interferon receptor signaling pathway can lead to the loss of IFN-γ's anti-tumor effect. Analysis of tumors of patients who were ineffective in treatment with ipilimumab, an anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4 antibody), showed that the mutation frequency of IFN-γ pathway genes interferon γ receptors 1 and 2 (IFNGR1 and IFNGR2), Janus kinase 2 (JAK2), and interferon regulatory factor 1 (IRF1) was high [6].
Any of these mutations will prevent IFN-γ signaling, allowing tumor cells to escape immune cell surveillance, leading to primary drug resistance. Mutations in this pathway can also lead to loss of PD-L1 expression after IFN-γ exposure, resulting in cancer cells being genetically negative for inducible PD-L1 expression. In this case, anti-PD-L1 targeted ICIs, which block the binding of PD-L1 and PD-1, show significant resistance [7]. Ling Fengyu's team at Yongchuan Hospital Chongqing Medical University revealed that approximately 50% of NSCLC patients develop secondary mutations after treatment with EGFR-TKIs, and there is still a huge need for effective treatment of EGFR mutant lung cancer. ICI inhibitors,

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represented by pembrolizumab, can be effective in previously treated PD-L1-positive NCSLC patients. Roy S Herbst's team at Yale University found that among 1034 patients, the mOS of the 2 mg/kg pembrolizumab group was 10.4 months, the mOS of the 10 mg/kg pembrolizumab group was 12.7 months, and the mOS of the docetaxel group was 8.5 months. The overall survival of the pembrolizumab group was significantly longer than that of the docetaxel group (hazard ratio [HR] 0.71, 95% CI 0.58-0.88; p=0.0008) [8]. The results of the study show that although pembrolizumab has a significant effect in the treatment of NCSLC patients, the emergence of drug resistance is still unavoidable. Studies have also found that pembrolizumab therapy may cause related liver damage. Due to the difficulty in detecting adverse drug reactions, it is necessary to add anticoagulants after excluding drug pathological reactions, and strengthen the postoperative and prognostic monitoring of patients.

EGFR mutations can also activate a variety of pro-survival signaling pathways (such as PI3K/AKT and mitogen-activated protein kinase (MAPK) pathways). These signaling pathways not only promote tumor cell proliferation and survival, but also induce the formation of an immunosuppressive microenvironment through a variety of mechanisms: upregulating the expression of immune checkpoint molecules such as PD-L1; promoting the recruitment of immunosuppressive cells such as MDSC and Treg; and inducing the secretion of immunosuppressive cytokines [9]. In addition, EGFR mutant tumors usually show a lower TMB, which means that they produce fewer neoantigens and are difficult to be recognized and attacked by the immune system. Since TMB is related to the number of neoantigens, low TMB means that fewer neoantigens can be recognized by T cells, thereby reducing the therapeutic effect of ICIs. This may be one of the core reasons for the poor therapeutic effect of ICIs in patients with EGFR mutations.

2) TME Factors

The immunosuppressive nature of the TME is one of the important reasons for the limited efficacy of ICI therapy. The TME plays a key role in the efficacy of ICI, especially involving cell plasticity and dedifferentiation processes, such as epithelial-mesenchymal transition (EMT), hypoxia, angiogenesis, and extracellular matrix (ECM) remodeling, which are closely related to the drug resistance of various cancer types.

Various immunosuppressive cell types in the TME also promote the occurrence of immune escape. In multiple preclinical models, Tregs, MDSCs, and immunosuppressive M2 macrophages have been shown to induce resistance to immune checkpoint blockade (ICB) therapy. Regarding the role of M2 macrophages in the TME, a study of preoperative biopsies of lung cancer patients showed that tumor tissues with low levels of PD-1+/CD8+T cell infiltration and high levels of M2 macrophage infiltration usually indicate poor response to PD-1/PD-L1 blockade therapy [9].

Within the tumor, continuous neoantigen-induced TCR signaling can cause T cells to enter a state of functional exhaustion, manifested by decreased cytotoxic activity and effector function. This "exhausted" T cell subset is often accompanied by high expression of multiple inhibitory immune checkpoint molecules. For example, in vitro studies have shown that tumor-infiltrating lymphocytes (TILs) often co-express multiple inhibitory molecules such as TIM-3 and LAG-3 on the basis of high PD-1 expression. For NSCLC patients with EGFR mutations, the infiltration density of TILs is generally low, suggesting that their tumors are in an "immune cold" state [9].

In addition, abnormal activation of EGFR signaling may further promote the enrichment of immunosuppressive cells (such as Tregs and MDSCs) and stimulate them to secrete immunosuppressive cytokines such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10), thereby weakening the anti-tumor immune response. The study also found that EGFR mutations may upregulate negative regulatory pathways including CTLA-4 and TIM-3, thereby offsetting the activation effect of the PD-1/PD-L1 pathway, suggesting that in the future, ICI combined with immune pathway inhibitors can be explored to improve efficacy.

ICI treatment shows anti-tumor activity and controllable safety, but its overall effectiveness still needs to be strengthened. Therefore, traditional chemotherapy is still a common treatment for EGFR mutants that develop resistance. However, the traditional dual-drug combined chemotherapy regimen as a second-line treatment after EGFR-TKIs resistance still cannot prevent the patient's tumor from continuing to progress, and the treatment effect is limited. From the perspective of existing ICIs and their application directions, the treatment range of ICIs has gradually expanded from advanced lung cancer to early lung cancer. Innovative strategies are needed to improve the efficiency of ICI treatment, which can provide potential cure opportunities for surgical patients.

III. Biomarkers to improve the inhibitory effect of EGFR mutations on PD-L1

ICI has made breakthrough progress in the treatment of advanced NSCLC and small cell lung cancer (SCLC), significantly extending the overall survival (OS) and pro-

gression-free survival (PFS) of patients. However, EGFR mutant tumors are insensitive to PD-L1 ICI immunosuppressants, which limits the efficacy of PD-L1-targeted ICI therapy. To expand the population eligible for ICI therapy, new biomarkers have been explored in recent years to mitigate the inhibitory effect of EGFR mutations on PD-L1.

A. Standard Biomarkers

The current biomarkers considered as the criteria for selecting the advantageous population for PD-L1 inhibitor treatment before the treatment include the following several types:

1) PD-L1 Expression

Currently, the expression of PD-L1 in tumor tissues is regarded as a biomarker for selecting the preferred population for PD-(L)1 inhibitor therapy. The results of the KEYNOTE-024 study showed that pembrolizumab had a better first-line treatment effect than chemotherapy in the advanced NSCLC population with a PD-L1 tumor proportion score of ≥50% and negative driver genes [10]. The KEYNOTE-042 study demonstrated that pembrolizumab could significantly improve the mOS of NSCLC patients with PD-L1 TPS ≥1% [11]. The CheckMate 057 study compared the efficacy of nivolumab monotherapy and docetaxel as second-line treatment for NSCLC. Regardless of the expression level of PD-L1, immunotherapy was beneficial compared to chemotherapy, but no similar OS benefit was observed in patients with low PD-L1 expression or undetectable PD-L1. Therefore, PD-L1 is one of the biomarkers for predicting the efficacy of immunotherapy in advanced NSCLC.

2) TMB

It refers to the relative number of gene mutations in a specific tumor tissue. Theoretically, the greater the number of mutations, the easier it is to stimulate the body's immunity. Multiple studies have indicated that high TMB is associated with a longer PFS. Based on these research results, the 2019 first edition of the National Comprehensive Cancer Network guidelines listed TMB as a biomarker for predicting the efficacy of nivolumab monotherapy or dual-drug combination immunotherapy for NSCLC. The exploratory analysis of CheckMate 026 suggested that patients with high TMB could benefit from immunotherapy. The exploratory analysis of the KEYNOTE series studies showed that regardless of the level of TMB, pembrolizumab combined with chemotherapy as first-line treatment could benefit the survival of NSCLC patients [12]. In order for TMB to become a reliable biomarker for efficacy prediction and be widely used in the future, several obstacles, including setting the cut-off value and adopting a unified analysis method, still need to be overcome.

3) DNA Mismatch Repair and Microsatellite Instability

The DNA mismatch repair (MMR) system can identify and repair nucleotide base mismatches, and is a key guardian of genomic integrity. This system is composed of multiple MMR proteins (MSH2, MSH3, MSH6, MLH1, PMS1, PMS2). If one or more of these MMR proteins are deficient in expression (deficient mismatch repair, dMMR), microsatellite instability (MSI) can occur. If no mutations are observed in the mismatch repair system, it is called proficient mismatch repair (pMMR), and it is mainly seen in microsatellite stable (MSS) cases [13]. The CheckMate 142 study showed that nivolumab monotherapy or nivolumab combined with ipilimumab has certain efficacy in treating MSI-H metastatic colorectal cancer. The incidence of dMMR/MSI-H in lung cancer is very low, so its predictive value for the efficacy of immunotherapy in lung cancer still needs further verification.

B. Newly Developed Biomarkers

The markers mentioned in the section are all tissue samples. Given the limitations of tissue samples in predicting the response to ICIs, in recent years, many studies have focused on finding new biological markers that can predict the efficacy of ICIs, in order to overcome the limitations of PD-L1 expression.

1) Infiltration of CD8+ T Cells

TILs, especially CD8+ T cells, are important predictors of the efficacy of ICI. Studies have shown that even in cases where PD-L1 expression is low, patients with high infiltration of CD8+ T cells may still respond to ICI. Tumors with EGFR mutations typically exhibit "cold tumor" characteristics (with less T cell infiltration), so enhancing the recruitment of CD8+ T cells may improve the efficacy of ICI.

2) Characteristics of IFN-γ-Related Genes

The activation of the IFN-γ signaling pathway (such as CXCL9, CXCL10, STAT1) can promote antigen presentation and T cell recruitment. The developed IFN-γ gene expression profile (GEP) score shows predictive value for ICI response in various cancers. EGFR mutations may inhibit the IFN-γ signal, so targeting this pathway may restore immune sensitivity.

3) Gut Microbiome

Recent studies have shown that the gut microbiota (such as Akkermansia muciniphila, Bifidobacterium) can modulate the immune treatment response. Certain bacterial groups may improve the efficacy of ICIs by enhancing the activation of DC cells or reducing the inhibitory effect of Tregs, but their role in patients with EGFR mutations still requires further investigation.

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4) Epigenetic Modifications (such as DNA Methylation)
The EGFR signal may inhibit the immune response through epigenetic silencing (such as PD-L1 methylation). Demethylating drugs (such as azacitidine) combined with ICI have shown a synergistic effect in preclinical models.
Furthermore, the combined use of multiple biomarkers can serve as comprehensive evidence, enhancing the predictive efficacy and accuracy, and better identifying patients who respond to immunotherapy, such as Tregs cells. Tregs have immunosuppressive effects and are associated with the low immune levels of patients with malignant tumors. They can suppress the immune response of the body by directly contacting cells and releasing inhibitory cytokines in the tumor microenvironment, thereby avoiding the occurrence of autoimmune diseases and maintaining immune homeostasis. De Simone et al. found that tumor-associated Tregs cells express specific molecules, such as interleukin-1 receptor type 2 (IL1R2), PD-L1/PD-L2, and chemokine receptor 8 (CCR8), on their surface, exerting immunosuppressive effects, and are negatively correlated with the prognosis of patients [14]. However, some studies have shown that high levels of Tregs are associated with a longer survival period for patients. Koh et al. found that in advanced NSCLC patients, the frequency of Tregs was higher in patients with longer PFS and OS, especially after anti-PD-1 treatment [15]. Compared with patients with low frequency of Tregs cells, there were significant differences in PFS and OS in patients with high frequency of Tregs cells. The reason for the increase in Tregs levels after anti-PD-1 treatment may be related to preventing the activation of the immune system and causing long-term excessive immunity. In addition, Tregs can exert immunoregulatory effects by releasing transforming growth factor-β (TGF-β). de Miguel-Perez et al. found that TGF-β is associated with non-response to ICIs and shorter PFS and OS, and TGF-β can promote tumor immune escape by increasing PD-L1 expression [16]. The above evidence indicates that the combination of Tregs and TGF-β may serve as a biomarker for predicting the efficacy of immunotherapy in NSCLC.

C. New ICI Combination Therapy

Based on the above information, by comparing the therapeutic effects of the new biomarkers with PD-L1, it can be observed that the newly developed biomarkers can improve the inhibitory effect of EGFR mutations on PD-L1. To expand the applicable population range of ICI therapy, the future optimization strategies for ICI treatment can include the following aspects: multi-biomarker combined detection: combining PD-L1, TMB, and CD8+ T cell infiltration indicators to improve the accuracy of patient screening; novel ICI combination therapy: such as the use of dual drugs like anti-PD-1/LAG-3 or anti-PD-1/TIM-3 to enhance the anti-tumor immune response; epigenetic regulation combined with ICI: for example, DNA methylating inhibitors may enhance T cell infiltration and improve the ICI response.

IV. conclusion

ICI has shown significant efficacy in the treatment of NSCLC, but resistance to ICI is prevalent in patients with EGFR mutations. The resistance mechanism involves both the intrinsic characteristics of tumor cells and the immunosuppressive nature of the tumor microenvironment (TME): on one hand, EGFR mutations lead to decreased MHC-I expression, abnormal IFN-γ signaling pathway, and reduced TMB; on the other hand, activation of the PI3K/AKT/MAPK pathway promotes PD-L1 expression, while there is insufficient infiltration of CD8+ T cells in the TME and enrichment of Tregs/MDSCs, creating an immunosuppressive environment. Moreover, the resistance of EGFR mutant patients to ICI limits its efficacy. The resistance mechanism mainly includes intrinsic factors of tumor cells (such as decreased MHC-I expression, abnormal IFN-γ signaling pathway) and immunosuppressive nature of the tumor microenvironment (such as Tregs, TGF-β secretion). To overcome resistance, future strategies should focus on combination therapy (such as ICI combined with anti-angiogenic drugs or epigenetic regulators), optimization of new biomarkers (such as TMB, CD8+ T cell infiltration and IFN-γ-related gene characteristics), dual ICBs (such as PD-1/LAG-3 combination), and exploration of neoadjuvant immunotherapy. By deeply analyzing the resistance mechanism and developing innovative therapies, it is expected to break through the ICI treatment bottleneck of EGFR mutant NSCLC and achieve more precise individualized treatment.

V. Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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