Immune Checkpoint Inhibitors in EGFR-Mutated NSCLC: Dusk or Dawn?

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ABSTRACT

Although immune checkpoint inhibitors (ICIs) that target programmed cell death protein-1/programmed cell death ligand-1 axis have significantly shifted the treatment paradigm in advanced NSCLC, clinical benefits of these agents are limited in patients with EGFR-mutated NSCLC. Several predictive biomarkers (e.g., programmed cell death ligand-1 expression, tumor mutation burden), which have been validated in EGFR-wild type NSCLC, however, are not efficacious in EGFR-mutated tumors, suggesting the unique characteristics of tumor microenvironment of EGFR-mutated NSCLC. Here, we first summarized the clinical evidence on the efficacy of ICIs in patients with EGFR-mutated NSCLC. Then, the cancer immunogram features of EGFR-mutated NSCLC was depicted to visualize the state of cancer-immune system interactions, including tumor foreignness, tumor sensitivity to immune effectors, metabolism, general immune status, immune cell infiltration, cytokines, and soluble molecules. We further discussed the potential subpopulations with EGFR mutations that could benefit from ICI treatment. Lastly, we put forward forward strategies to adequately maximize the efficacy of ICI treatment in patients with EGFR-mutated NSCLC in the upcoming era of combination immunotherapies.

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Keywords: Immune-checkpoint inhibitor; Epidermal growth factor receptor; Tumor microenvironment; Non–small cell lung cancer; PD-1/PD-L1 inhibitor

Introduction

EGFR mutation is the most often detected oncogenic driver in Asians with 50% incidence and 10% to 15% incidence in westerners with lung adenocarcinoma (LUADs).1,2 A number of clinical trials revealed that EGFR-tyrosine kinase inhibitors (TKIs) have superior therapeutic effect compared with chemotherapy in patients with advanced NSCLC with EGFR-sensitive mutations. First-generation (erlotinib, gefitinib, and icotinib) or second-generation (afatinib) TKIs had been found to have a median progression-free survival (mPFS) of 9 to 13 months and third-generation (osimertinib) EGFR TKI with 18.9 months.3–6 Therefore, EGFR TKI is currently the first-line standard of care for these patients. Nevertheless, resistance to EGFR TKIs is inevitable and innovative therapeutic strategies afterward still remain an unresolved issue.7

In contrast with EGFR TKIs, immune checkpoint inhibitors (ICIs) targeting programmed cell death protein-1 (PD-1) and programmed death ligand-1 (PD-L1) were designed to release the brakes on T cells and have been found to have promising antitumor efficacy in advanced NSCLC.8–13 A series of preclinical and retrospective studies have suggested that activating EGFR mutations

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could up-regulate PD-L1 expression in NSCLC, which is a predictive biomarker of response to PD-1/PD-L1 blockade. In addition, preclinical results further revealed that PD-1 blockade improved the survival of mice with EGFR-driven lung adenocarcinomas by enhancing function of effector T cells and decreasing the levels of protumorigenic cytokines. Despite the preclinical evidence, patients with EGFR-mutated NSCLC had poor response to ICIs. A meta-analysis of several trials on PD-1/PD-L1 inhibition in second-line treatment of NSCLC and two large retrospective analyses reported that ICIs do not improve OS in patients with EGFR-mutated NSCLC. In addition to their failure as monotherapy treatments, the combination of ICI and TKIs did not result in a synergistic antitumor effect in patients but induced high incidence of toxicity.

Recently, researchers are attempting to reveal the possible underlying mechanisms for the poor efficacy of anti–PD-1/PD-L1 treatment in EGFR-mutated NSCLC. Several studies have revealed that immunosuppressive tumor microenvironment (TME), tumor mutation burden (TMB), and PD-L1 expression were possible explanations for poor immunoresponse. Besides, preclinical and clinical studies revealed that initial EGFR TKIs could induce antitumor immunity through various ways. Nevertheless, because the factors mentioned previously are spatially and temporally variable and influenced by multiple complex interactions, the specific correlation between them is still unclear and requires further research. In this review, we summarized the clinical data with regard to the efficacy of ICI in patients with EGFR-mutated NSCLC and deciphered the unique TME in EGFR-mutated NSCLC. We further delineated the specific subpopulation in EGFR-mutated NSCLC that possibly benefits from immunotherapy and put forward future strategies to improve the efficacy of immunotherapy in EGFR-mutated NSCLC.

**Clinical Data of ICIs in EGFR-Mutated NSCLC**

**Efficacy of ICI Monotherapy**

KEYNOTE-001 started the explorations on efficacy of ICIs on EGFR-driven patients as first-line treatment strategy. The data revealed that four EGFR TKI–naïve patients treated with pembrolizumab had improved clinical outcome with 50% of objective response rate (ORR), 157.5 days of mPFS, and 559 days of median OS (mOS). A total of 26 EGFR-mutated patients who had received EGFR TKI before administration of pembrolizumab had limited response (ORR = 4%, mPFS = 56 d, mOS = 120 d). Despite of the small sample size, it indeed formed the basis for the following phase 2 trial of pembrolizumab in TKI-naïve patients with PD-L1–positive expression. Nevertheless, none of 11 patients got clinical benefit from pembrolizumab. CheckMate 012 also revealed that nivolumab monotherapy revealed poor efficacy in patients with EGFR mutations compared with EGFR-wild type (ORR: 14% versus 30%; mPFS: 1.8 versus 8.8 mo; mOS: 18.8 mo versus not reached). In the second or subsequent line, immunotherapy alone also seemed to be ineffective in patients with EGFR-mutated NSCLC as revealed in CheckMate 057, OAK, POPLAR, and KEYNOTE-010. The finding of no OS benefit in patients with EGFR-mutated NSCLC in two pooled meta-analyses further suggested that the application of immunotherapy in patients with EGFR-mutated NSCLC should only be referred only if no effective therapies were present. BIRCH study was designed to evaluate the efficacy of atezolizumab in different lines for patients with PD-L1–selected advanced NSCLC. A total of 45 patients with EGFR mutations were included. Although the results revealed that atezolizumab had antitumor activity both in EGFR-mutated and EGFR-wild type tumors, atezolizumab had less effectiveness compared with wild-type tumors, which was consistent with previous data.

Collectively, data from these clinical trials (Table 1) possibly prompted us that EGFR-mutated NSCLC had poor response to PD-1/PD-L1 monotherapy and patients with EGFR-mutated NSCLC with high PD-L1 expression could be potential benefit population.

**Efficacy of ICI-Based Combination Therapy**

**Combining With Chemotherapy.** In CheckMate 012, 6 patients with EGFR mutations were enrolled to evaluate the efficacy of combination of nivolumab and chemotherapy in the first-line setting. Nevertheless, mPFS and OS were shorter in EGFR-mutated group than that in wild-type group (mPFS: 4.8 versus 7.5 mo, mOS: 20.5 versus 24.5 mo). In 2019 World Conference on Lung Cancer (WCLC), our group reported a phase 2 study of toripalimab plus chemotherapy with promising antitumor efficacy in patients with EGFR-mutated advanced NSCLC who had progressed after EGFR TKI treatment, with an ORR of 50% and median PFS of 7.0 months. Several ongoing phase 3 trials are aiming to evaluate the efficacy of ICI plus chemotherapy in patients with EGFR-mutated NSCLC who progressed on previous TKI (CheckMate-722, KEYNOTE-789, NCT03924050).
<table>
<thead>
<tr>
<th>Name of Study</th>
<th>Phase</th>
<th>No. of EGFR(+) Patients</th>
<th>Treatment</th>
<th>Treatment Line</th>
<th>ORR</th>
<th>mPFS</th>
<th>mOS</th>
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<td><strong>Monotherapy</strong></td>
<td></td>
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</tr>
<tr>
<td>KEYNOTE001</td>
<td>1</td>
<td>30</td>
<td>Pembrolizumab</td>
<td>First-line TKI naive: 50%</td>
<td>ORR: 50%</td>
<td>mPFS: 157.5 d</td>
<td>mOS: 559 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TKI pretreated: 4%</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CheckMate012</td>
<td>1</td>
<td>7</td>
<td>Nivolumab</td>
<td>First-line TKI naive: 14%</td>
<td>ORR: 14%</td>
<td>mPFS: 1.8 mo</td>
<td>mOS: 20.1 mo</td>
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<td>NCT02879994</td>
<td>2</td>
<td>11</td>
<td>Pembrolizumab</td>
<td>First-line TKI naive: 4%</td>
<td>ORR: 4%</td>
<td>mPFS: 1.8 mo</td>
<td>mOS: 18.8 mo</td>
</tr>
<tr>
<td>BIRCH</td>
<td>2</td>
<td>Cohort 1 (1L): 13</td>
<td>Atezolizumab</td>
<td>Across lines Cohort 1 (1L): 23%</td>
<td>ORR: 23%</td>
<td>mPFS: 1.3 mo</td>
<td>mOS: 20.1 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cohort 2 (2L): 18</td>
<td></td>
<td>Cohort 2 (2L): 0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cohort 3 (3L): 14</td>
<td></td>
<td>Cohort 3 (3L): 7%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>POPLAR</td>
<td>2</td>
<td>19</td>
<td>Atezolizumab</td>
<td>Second or subsequent</td>
<td>ORR: 11%</td>
<td>mPFS: 5.5 mo</td>
<td>mOS: 20.1 mo</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td>KEYNOTE010</td>
<td>2/3</td>
<td>86</td>
<td>Pembrolizumab</td>
<td>Second or subsequent</td>
<td>ORR: 5%</td>
<td>mPFS: 1.7 mo</td>
<td>mOS: 7.4 mo</td>
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<td>OAK</td>
<td>3</td>
<td>85</td>
<td>Atezolizumab</td>
<td>Second or subsequent</td>
<td>ORR: 11%</td>
<td>mPFS: 1.3 mo</td>
<td>mOS: 7.4 mo</td>
</tr>
<tr>
<td>CheckMate057</td>
<td>3</td>
<td>82</td>
<td>Nivolumab</td>
<td>Second or subsequent</td>
<td>ORR: 11%</td>
<td>mPFS: 1.3 mo</td>
<td>mOS: 7.4 mo</td>
</tr>
<tr>
<td>ATLANTIC</td>
<td>2</td>
<td>102</td>
<td>Durvalumab</td>
<td>Third or subsequent</td>
<td>ORR: 11%</td>
<td>mPFS: 1.3 mo</td>
<td>mOS: 7.4 mo</td>
</tr>
</tbody>
</table>

| **Combination therapy** |       |                         |                            |                |     |               |         |
| **Combined with chemotherapy** |       |                         |                            |                |     |               |         |
| CheckMate012        | 1     | 6                       | Nivolumab + chemotherapy   | First-line TKI naive: 50% | ORR: 50%  | mPFS: 157.5 d | mOS: 559 d |
| NCT03513666         | 2     | 40                      | Toripalimab + chemotherapy | First-line TKI naive: 4% | ORR: 4%   | mPFS: 157.5 d | mOS: 559 d |

| **Combined with CTLA-4 blockade** |       |                         |                            |                |     |               |         |
| CheckMate012        | 1     | 8                       | Nivolumab + ipilimumab     | First-line TKI naive: 50% | ORR: 50%  | mPFS: 157.5 d | mOS: 559 d |
| KEYNOTE021          | cohort D and H | 10 | Pembrolizumab + ipilimumab | First-line TKI naive: 4% | ORR: 4%   | mPFS: 157.5 d | mOS: 559 d |

| **Combined with antiangiogenesis** |       |                         |                            |                |     |               |         |
| IMpower150          | 3     | 124                     | Atezolizumab + bevacizumab | Second or subsequent | ORR: 50%  | mPFS: 157.5 d | mOS: 559 d |
|                     |       |                         | Atezolizumab + chemotherapy | First-line TKI naive: 4% | ORR: 4%   | mPFS: 157.5 d | mOS: 559 d |
|                     |       |                         | (ABCP group, n = 34)       |                |     |               |         |
|                     |       |                         | Atezolizumab + chemotherapy | First-line TKI naive: 4% | ORR: 4%   | mPFS: 157.5 d | mOS: 559 d |
|                     |       |                         | (ACP group, n = 45)       |                |     |               |         |
|                     |       |                         | Atezolizumab + chemotherapy | First-line TKI naive: 4% | ORR: 4%   | mPFS: 157.5 d | mOS: 559 d |
|                     |       |                         | (BCP group, n = 45)       |                |     |               |         |

(continued)
<table>
<thead>
<tr>
<th>Name of Study</th>
<th>Phase</th>
<th>No. of EGFR(+) Patients</th>
<th>Treatment</th>
<th>Treatment Line</th>
<th>ORR</th>
<th>mPFS</th>
<th>mOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined with EGFR TKI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CheckMate012</td>
<td>1</td>
<td>21</td>
<td>Nivolumab + erlotinib</td>
<td>First-line (1 pt) second or subsequent (20 pts)</td>
<td>15%</td>
<td>—</td>
<td>—</td>
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<tr>
<td>NCT02088112</td>
<td>1</td>
<td>40</td>
<td>Durvalumab + gefitinib</td>
<td>First-line (30 pts) second (10 pts)</td>
<td>TKI naive: 63.3% TKI pretreated: 70.0%</td>
<td>TKI naive: 10.1 mo TKI pretreated: 12.0 mo</td>
<td>—</td>
</tr>
<tr>
<td>TATTON</td>
<td>1b</td>
<td>23</td>
<td>Durvalumab + osimertinib</td>
<td>Second or subsequent</td>
<td>43%</td>
<td>—</td>
<td>—</td>
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<tr>
<td>NCT02013219</td>
<td>1b</td>
<td>28</td>
<td>Atezolizumab + gefitinib</td>
<td>First-line</td>
<td>75%</td>
<td>11.3 mo</td>
<td>—</td>
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<tr>
<td>KEYNOTE021 cohort E and F</td>
<td>1/2</td>
<td>19</td>
<td>Pembrolizumab + erlotinib (n = 12)/gefitinib (n = 7)</td>
<td>First-line</td>
<td>41.7%</td>
<td>14.3% erlotinib: 19.5 mo gefitinib: 1.4 mo</td>
<td>—</td>
</tr>
<tr>
<td>CAURAL</td>
<td>3</td>
<td>14</td>
<td>Durvalumab + osimertinib</td>
<td>Second or subsequent</td>
<td>60%</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Other clinical data

Meta-analysis

Lee et al.21 | — | 186 | Anti-PD-1/PD-L1 vs. docetaxel | Second or subsequent | — | — | HR 1.05 (0.70, 1.55) |
Lee et al.32 | — | 271 | Anti-PD-1/PD-L1 vs. docetaxel | Second or subsequent | — | — | HR 1.11 (0.80, 1.53) |

Large retrospective analysis

Italian EAP | — | 102 | Nivolumab | Second or subsequent | 8.8% | 3.0 mo | 8.3 mo |
IMMUNOTARGET | — | 125 | All immune checkpoint inhibitors | Across lines | 12% | 2.1 mo | 10 mo |

1L, first-line; 2L, second-line; 3L, third-line; ABCP, atezolizumab + bevacizumab + carboplatin + pemetrexed; ACP, atezolizumab + carboplatin + pemetrexed; BCP, bevacizumab + carboplatin + pemetrexed; HR, hazard ratio; mOS, median overall survival; mPFS, median progression-free survival; NA, not applicable; NE, not estimable; ORR, overall response rate; PD-1, programmed cell death protein-1; PD-L1, programmed death ligand-1; TKI, tyrosine kinase inhibitor.
Combining With CTLA-4 Blockade. CheckMate 012 also enrolled eight patients with EGFR mutation to receive nivolumab combining with ipilimumab as first-line treatment strategy.47 Four of eight patients (50%) achieved objective response whereas ORR was 41% in patients with EGFR-wild type. In KEYNOTE 021-cohorts D and H, 10 EGFR TKI-pretreated patients with EGFR mutation were enrolled to receive pembrolizumab 2 mg/kg and ipilimumab 1 mg/kg. Only one patient (10%) had objective response.48

Combining With Antiangiogenesis. The IMPOWER 150 uncovered the crucial role of antiangiogenic agents in EGFR-mutated NSCLC. Updated data reported in 2020 European Society for Medical Oncology (ESMO) revealed that patients who received PD-L1 blockade and antiangiogenesis plus chemotherapy had better clinical outcome with an ORR of 73.5% (versus 40.9% in bevacizumab plus chemotherapy group) and mPFS of 10.2 months (versus 7.1 mo in bevacizumab plus chemotherapy group).39 Several studies are underway to investigate the role of ICIs combined with antiangiogenesis in patients with EGFR-mutated NSCLC after failure of previous EGFR TKIs (NCT04099836, NCT04116918, NCT04120454).

Data from ICI-based combination trials revealed that combination therapy with chemotherapy or antiangiogenesis might improve the clinical outcomes in patients with EGFR-mutated NSCLC who progressed on previous EGFR TKI (Table 1) and several ongoing trials are evaluating ICI-based treatment in this population (Table 2).

Toxicity Issues

On the basis of the previous evidence, ICI either as monotherapy or in combination with chemotherapy or antiangiogenesis therapy was generally well tolerated in patients with EGFR-mutated NSCLC, and no new safety signals were observed. Nevertheless, immune-related toxicity issues should be noted in patients with EGFR-mutated NSCLC who received ICIs plus EGFR TKIs.

The phase 1b TATTON study was designed to evaluate the safety and tolerability of combining osimertinib and durvalumab in patients with EGFR-mutated NSCLC who progressed on previous TKI.24 Nevertheless, the incidence of interstitial lung disease was 38%, which was higher than expected. Therefore, the recruitment of the phase 3 trial, CAURAL, was suspended.40 Similarly, although concurrent durvalumab plus gefitinib or induction of gefitinib before administration of durvalumab and gefitinib revealed antitumor activity in EGFR-mutated NSCLC,41 unfortunately, these combinations also resulted in high discontinuation rate owing to liver toxicity. Another phase 1b study evaluating safety and clinical activity of atezolizumab and gefitinib reported 75% of ORR. Although no pneumonitis was observed, treatment-related serious adverse events (AEs) occurred in 50% of patients.42 In KEYNOTE 021, 12 TKI-naive patients received pembrolizumab plus erlotinib and seven received pembrolizumab and gefitinib.43 Pembrolizumab plus erlotinib was feasible with 41.7% of ORR and manageable safety profile. Nevertheless, high-grade liver toxicity was observed in 71.4% of patients.

In addition, recent studies informed that treatment sequence of immunotherapy and EGFR TKI also had impact on the incidence of treatment-related toxicity. A recent database analysis revealed a higher proportion of EGFR TKI-associated interstitial pneumonitis in nivolumab-treated patients with NSCLC.44 A retrospective analysis of 41 patients who were treated with sequential PD-1/PD-L1 inhibitors followed by osimertinib found that 15% of patients developed a severe immune-related AEs (irAEs), especially in those who began osimertinib within 3 months (5 of 21, 24%) of previous PD-1/PD-L1 inhibitors compared with those more than 3 to 12 months (1 of 8, 13%) or more than 12 months (0 of 12, 0%). In addition, no severe irAEs were observed among patients treated with PD-1/PD-L1 blockade followed by other EGFR TKIs (afatinib or erlotinib).45 To confirm this clinical observation, our group used the doxycycline-inducible transgenic EGFR<sup>L858R</sup> mouse model to investigate whether combination of gefitinib or osimertinib and anti-PD-L1 would cause lung injury. We found that combination of osimertinib rather than gefitinib led to severe lung injury, especially in anti-PD-L1 followed by osimertinib group with increased proinflammatory cytokines.46 Therefore, in current clinical practice, switching regimen from an ICI to EGFR TKI, especially osimertinib, should be administrated with caution, as unpredictable severe irAEs may occur.

Hyperprogressive Disease on ICI Treatment

Hyperprogressive disease (HPD) was observed in a subset of patients who experienced rapid tumor growth and clinical deterioration after ICI. It was often defined as (1) time to treatment failure (TTF) less than 2 months; (2) more than 50% increase in tumor size compared with pretherapy; and (3) more than two-fold increase in progression rate.47 The incidence of HPD after ICI treatment in NSCLC ranged from 8% to 14%.47,48 EGFR mutations were reported to be potentially related to HPD. Kato et al.47 noted that EGFR alterations and MDM2 amplification were potentially related to HPD in patients treated with ICIs. A total of 8 of 10 patients with EGFR alterations had TTF less than 2 months, and two patients had 36-fold and 42-fold
<table>
<thead>
<tr>
<th>Clinical Trial</th>
<th>Phase</th>
<th>Treatment Line</th>
<th>Combo Classification</th>
<th>Experimental Arm</th>
<th>Enrolled Population</th>
<th>Status</th>
<th>Estimated Study Completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT04120454</td>
<td>2</td>
<td>2+ ICI + antiangiogenesis</td>
<td>Pembrolizumab + ramucirumab</td>
<td>Recurrent or metastatic NSCLC with sensitizing EGFR mutations</td>
<td>Recruiting</td>
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<td>NCT04099836</td>
<td>2</td>
<td>2+ ICI + antiangiogenesis</td>
<td>Atezolizumab + bevacizumab</td>
<td>Stage IV EGFR-mutant NSCLC with progression on osimertinib</td>
<td>Recruiting</td>
<td>November 10, 2025</td>
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<tr>
<td>NCT04116918</td>
<td>2</td>
<td>2+ ICI + antiangiogenesis</td>
<td>JS001 + anlotinib</td>
<td>EGFR TKI-resistant T790M-negative NSCLC patients</td>
<td>Recruiting</td>
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<tr>
<td>NCT04517526</td>
<td>2</td>
<td>2+ ICI + antiangiogenesis</td>
<td>Durvalumab + pemetrexed + cis/cis/cis + carboplatin + bevacizumab (+ SBRT for subsequent oligometastatics)</td>
<td>Stage IV EGFR-mutant NSCLC after failure of first-line osimertinib</td>
<td>Not yet recruiting</td>
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<td>NCT04405674</td>
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<td>Tislelizumab + carboplatin/nab-paclitaxel (pemetrexed as maintenance)</td>
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<td>1/1b</td>
<td>2+ ICI + EGFR TKI</td>
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<td>advanced or metastatic EGFR-mutated NSCLC with progression on erlotinib</td>
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<tr>
<td>NCT03786692</td>
<td>2</td>
<td>2+ ICI + chemo + antiangiogenesis</td>
<td>Atezolizumab + pemetrexed + carboplatin + bevacizumab</td>
<td>Stage IV nonsquamous NSCLC patients with sensitizing EGFR mutation or nonsmokers</td>
<td>Recruiting</td>
<td>January 2024</td>
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<tr>
<td>NCT03994393 (ILLUMINATE)</td>
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<td>2+ ICI + ICI</td>
<td>Durvalumab + tremelimumab</td>
<td>Metastatic NSCLC with EGFR-sensitizing mutation after failure of EGFR TKI therapy (T790M+/−)</td>
<td>Recruiting</td>
<td>November 2021</td>
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<tr>
<td>NCT03944772 (ORCHARD)</td>
<td>2</td>
<td>2+ ICI + chemo</td>
<td>Experimental module 4: durvalumab + pemetrexed + carboplatin</td>
<td>Stage IV EGFR-mutant NSCLC after failure of first-line osimertinib (biomarker-guided)</td>
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<td>NCT03242915</td>
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<td>August, 2022</td>
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<td>NCT03944772 (KEYNOTE-789)</td>
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<td>2+ ICI + chemo</td>
<td>Pembrolizumab + pemetrexed + carboplatin/cisplatin</td>
<td>Metastatic nonsquamous EGFR-mutated NSCLC with progression on previous TKI therapy</td>
<td>Active, not recruiting</td>
<td>June 15, 2023</td>
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<td>NCT02864251</td>
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<td>2+ ICI + chemo</td>
<td>Nivolumab + pemetrexed + cisplatin/cisplatin</td>
<td>Stage IV EGFR-mutant NSCLC after failure of first/second-line of TKI therapy</td>
<td>Active, not recruiting</td>
<td>July 14, 2022</td>
<td></td>
</tr>
<tr>
<td>NCT03802240</td>
<td>3</td>
<td>2+ ICI + chemo + antiangiogenesis</td>
<td>Sintilimab + IBI305 + pemetrexed + cisplatin</td>
<td>TKI-resistant EGFR-mutated Chinese nonsquamous patients with NSCLC</td>
<td>Recruiting</td>
<td>May 31, 2021</td>
<td></td>
</tr>
</tbody>
</table>

Chemo, chemotherapy; ICI, immune checkpoint inhibitor; SBRT, stereotactic body radiation therapy; TKI, tyrosine kinase inhibitor.
increase in progression pace compared with pretreatment from documented imaging data. Nevertheless, the subsequent study performed by Ferrara et al. reported that none of 16 patients with EGFR-mutated NSCLC experienced HPD, but whether the other alterations in EGFR had impact on incidence of HPD was not mentioned. In 2017 ESMO, Singavi et al. reported that EGFR amplifications might lead to HPD; however, Kim et al. did not find that EGFR amplifications or mutations were associated with HPD. Therefore, the role of distinct EGFR alterations in driving HPD or if it has correlations with HPD remains unclear. The interplay between ICI resistance and acceleration of tumor growth remained undetermined in EGFR-mutated NSCLC. There are two possible explanations: (1) Blockade of PD-1/PD-L1 axis could potentially facilitate the expansion of PD-1 plus regulatory T-cell (Treg) cells in HPD to form an immunosuppressive TME. On the basis of the previous evidence, Treg cells were accumulated in EGFR tumors. Therefore, to clarify the numbers of PD-1 plus Treg cells helps identify the mechanisms of HPD in patients with EGFR-mutated NSCLC. (2) The other possible explanation could be that PD-1/PD-L1 interaction suppressed oncogenic signaling pathway in tumor cells by enhancing PTEN-dependent signaling and inhibiting phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) or NF-κB signaling. Oncogenic pathway was hyperactivated in EGFR-mutated tumors; therefore, unleashed brakes of PD-1/PD-L1 axis led to hyper-reactivation of oncogenic pathway, resulting in rapid acceleration of EGFR-mutated tumor cells.

Immunogram Features of EGFR-Mutated NSCLC

Blank et al. proposed the “cancer immunogram” to describe the cancer-immune interactions with the aim to focus on biomarker research and may spur the personalized therapy. We integrated the seven parameter classes with the features of TME in EGFR-mutated NSCLC to constitute a framework that could partly uncover the underlying mechanisms for poor immunotherapy response in such population (Fig. 1).

Checkpoint: PD-L1 Expression

Basically, interactions between PD-L1 on tumor cells and PD-1 on T cells impede T cell-mediated antitumor activity. ICIs targeting PD-1/PD-L1 are supposed to restore the cytotoxic activity of T cells. Nevertheless, given the complex nature of EGFR-mutated tumors, the importance of PD-1/PD-L1 axis should be addressed throughout the components in TME (Fig. 2). Although many experimental data suggested that EGFR mutations could up-regulate PD-L1 expression by means of five possible pathways, such as Ras/RAF/MEK/ERK, PI3K/AKT/mTOR, JAK/STAT, NF-κB, and YAP, epidemiologic relationship between EGFR mutations and PD-L1 expression still remains controversial. Azuma et al. first reported that high expression of PD-L1 was associated with the presence of EGFR mutations in surgically resected NSCLC. Nevertheless, several recent studies revealed the negative correlation between EGFR mutations and PD-L1 expression. Similarly, Dong

Figure 1. The cancer immunogram. The plot combines the seven parameters that characterize cancer-immune interactions with features in EGFR-mutated NSCLC. HLA, human leukocyte antigen; IL-6, interleukin-6; IL-8, interleukin-8; MDSC, myeloid-derived suppressor cell; NK, natural killer; PD-L1, programmed death ligand-1; TAN, tumor-associated neutrophil; TCR, T cell receptor; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.
et al. analyzed 3283 patients from 15 studies and found that the high expression of PD-L1 was more likely to be detected in patients with EGFR-wild type. They further evaluated mRNA and protein levels of PD-L1 in resected NSCLC tissues from The Cancer Genome Atlas (TCGA) and internal database (Guangdong Lung Cancer Institute [GLCI]) and found that the PD-L1 expression was lower in EGFR-mutated tumors than in EGFR-wild type tumors. The conflicting results of different studies could be related to the heterogeneity of the study populations, variant patient tumor tissue resources, distinct PD-L1 testing platform, different PD-L1 antibodies, and tumor proportion score cutoff values used in PD-L1 evaluation. In addition, both genetic alterations and inflammation, such as interferon (IFN)-γ, could induce PD-L1 expression. Thus, even though EGFR-mutated NSCLC cell lines exhibited slightly higher CD274 (encoding PD-L1) expression than EGFR wild-type NSCLC cell lines, IRF1 suppressed by EGFR signaling in EGFR-mutated lung adenocarcinoma may decrease the IFN-γ level and result in a low increase in CD274 expression in resected tumor specimens, which could possibly explain the controversies between preclinical data and clinical results. Besides, the stability of PD-L1 may be also a confounding factor contributing to the inconsistent results, for it was observed in breast cancer that the activation of EGFR signaling pathway inactivated glycogen synthase kinase 3β and thereby stabilized PD-L1 expression. Thus, the association of PD-L1 expression and EGFR mutation is not available yet and still needs further investigations.

**Tumor Foreignness: TMB**

TMB was defined as the number of somatic mutations per mega base in the coding region of the tumor genome. Tumor neoantigen is the repertoire of peptide that could be recognized by T cell receptor (TCR) by means of presented by major histocompatibility complex (MHC) molecules and, thus, induces antitumor T cell responses. Nevertheless, tumors harboring EGFR mutation had lower somatic mutations and lower number of neoantigens because patients with EGFR-mutated NSCLC were often found in never smokers. The TMB data from TCGA revealed that mutational burden in EGFR-mutated group...
was comparatively lower than EGFR-wild type (median TMB: 56 versus 181, p < 0.001). In another two data sets (Broad and GLCI), EGFR-mutated group also had lower TMB (Broad: 59 versus 209, p = 0.003; GLCI: 162 versus 197, p = 0.029).67 Nevertheless, Voke et al.74 reported that TMB is not associated with response to ICI if driver mutations were present. Therefore, whether the low TMB accounts for the poor response to ICI in EGFR-mutated NSCLC should be further investigated.

Tumor Sensitivity to Immune Effectors: Human Leukocyte Antigen-TCR Axis

Human leukocyte antigen (HLA) class I heterozygosity was associated with ICI efficacy.75 EGFR-mutated tumors had lower numbers of candidate MHC-I and MHC-II neoantigens compared with EGFR/KRAS wild type or KRAS-mutated tumors.76 Nevertheless, Negrao et al.77 collected the HLA typing and genomic and clinical data from patients treated with ICI and found no correlation between ICI benefit and HLA class I zygosity. Instead, the presence of targetable driver mutations predicted the worse clinical outcome of ICIs. Therefore, HLA heterozygosity might not be an independent predictive marker for immunotherapy in EGFR-mutated tumors.

The number of nonsynonymous mutations was positively related to T cell clonality. Previous studies revealed that TCR repertoire was an indicator for immune monitoring across many tumors.78,79 In melanoma, patients with greater T cell clonal expansion had improved clinical outcomes treated with ICIs.80 EGFR-mutated tumors were associated with higher richness, lower T cell clonality, and similar density of T cell repertoire compared with EGFR wild-type tumors.81 Intriguingly, both in low TMB subgroup, T cell clonality was still higher in EGFR-wild type tumors. These results indicated that EGFR-mutated tumors had impaired ability to induce better T cell clonality. This could partly explain the reason why patients with EGFR-mutated NSCLC with high PD-L1 expression still had lower response to immunotherapy.

TME is a very complex system to support tumor growth that includes malignant cells, immune cells, stroma, fibroblast, cytokines, and so on. Activation of EGFR signaling pathway plays a crucial role in tumor genesis and tumor development and could influence multiple compartments in TME (Fig. 3).

Tumor Metabolism: Adenosine Signaling Pathway

Adenosine signaling axis is thought to produce broad immunosuppressive effect on TME, including suppression of natural killer (NK) and CD8+ T cell cytolytic activity, enhancement of suppressive macrophage function, and proliferation of Tregs and myeloid-derived suppressor cell (MDSC).82,83 CD73 is ecto-5’-nucleotidase encoded by NT5E gene and a pivotal enzyme that converts AMP to adenosine. Our previous study suggested that CD73 was related to immune suppression and poor prognosis in various cancers.84 Preclinical studies revealed that inhibition of CD73 resulted in improved immune response, suggesting CD73 as a potential treatment target. Streicher et al.85 noted that EGFR-mutated cell lines had significantly higher CD73 mRNA and protein expression compared with EGFR wild-type cell lines. Furthermore, CD73 expression could be induced by EGF and decreased by EGFR inhibitor. In addition, Park et al.86 found that CD73 expression was significantly increased in EGFR-mutated samples. Similarly, Le et al.87 found that top up-regulated genes in EGFR-mutated tumors including NT5E and ADORA1 belonged to CD73/adenosine pathway. In the mouse model of EGFR-mutated lung cancer, CD73 blockade significantly inhibited tumor growth.87 Therefore, it was hypothesized that increased CD73 may contribute to low response rates to immunotherapy in EGFR-mutated NSCLC and, possibly, CD73/adenosine pathway could be served as the therapeutic target for EGFR-mutated NSCLC.86 Nevertheless, Ishii et al.88 revealed that in patients with EGFR-mutated NSCLC, high CD73 expression was related to better efficacy to anti-PD1 compared with low expression (ORR: 66.7% versus 0%, PFS: 16.0 versus 1.2 mo, p = 0.02). There was no statistical significance on PFS between EGFR-mutated and wild-type groups.85

CD39 is an integral membrane protein that could degrade ATP or ADP to yield AMP. It is the marker for exhausted CD8+ T cells and a marker indicating tumor-specific CD8+ T cells.89 Functional exhausted T cells could be reinvigorated by PD-1/PD-L1 inhibitors. Therefore, high expression of CD39 could be a predictive marker for immunotherapy.90 To support this hypothesis, a recent study reported that an expansion of CD39+ population in the peripheral blood was associated with response to PD-1 blockade.91 Lack of CD39 could be related to cancer-unrelated bystander CD8+ tumor-infiltrating lymphocytes (TILs). Simoni et al.91 reported that the frequency of CD39+CD8+ TILs was significantly higher in patients with EGFR-wild type tumors, which was consistent with our results reported in 2020 WCLC. Our preliminary results found that treatment-naive patients with EGFR-mutated tumors had fewer proportions of CD8+CD39+ T cells in peripheral blood. CD39+-expressing CD8+ T cells were significantly increased in the presence of EGFR-WT cell lines than EGFR-mutated cell lines. In addition, CD8+CD39+ T cells were correlated with functional state of T cells and immunotherapy response.92 Therefore, the poor observed response to PD-1 blockade could be possibly related to the abundance of bystander CD39-CD8+T
cells in EGFR-mutated tumors. Another study pointed out that CD39 was a promising marker in patients with EGFR-mutated NSCLC and associated with longer disease-free survival and inflamed TME.93 Hence, the role of CD73 or CD39 is not simple as we have noted as the role in adenosine signaling pathway could have multiple connections in TME. Uncovering the exact roles of CD39 and CD73 is very important to clarify the complicated TME in patients with EGFR-mutated NSCLC.

**General Immune Status: TILs**

TILs are crucial cell populations that can infiltrate both tumor nests and stroma. TILs act as soldiers to exert killing function. Higher density of CD8+ TILs was associated with better clinical outcome.94,95 A previous study reported that there was no statistical significance in density of CD8+ T cells between EGFR-mutated and wild-type tumors.96 Nevertheless, other investigations uncovered that EGFR-mutated tumors were characterized with few TILs or absence of TILs. Previously, TME was classified into three groups on the basis of the expression of CD3, Ki67, and granzyme B (group 1, low CD3+ expression; group 2, high CD3 expression with low expression of Ki67 or cytotoxicity Granzyme B; group 3, high CD3 expression with high proliferation Ki67 or cytotoxicity Granzyme B).
though abundant TILs were present, high proportions of inactivated status were observed (group 2) compared with EGFR-wild type or KRAS-mutated.\textsuperscript{67} Another study reported that EGFR-mutated cases had lower levels of T-cell infiltration but the expression of Granzyme B and Ki-67 in T cells was not different from tumors with KRAS mutation or tumors without EGFR or KRAS mutations.\textsuperscript{76}

On the basis of the four types of TME according to the expression of CD8\textsuperscript{+} TILs and PD-L1, evidence suggested that EGFR-mutated tumors lack CD8\textsuperscript{+} TIL infiltration and uninfamed TME with lower proportions of type I tumors (PD-L1+/CD8+TILs+).\textsuperscript{67,98}

Regarding CD8\textsuperscript{+} TIL infiltration, a series of studies reported the inconsistent results. Multiple studies used next-generation sequencing with RNA sequencing data to calculate the density of CD8\textsuperscript{+} cells. Nevertheless, immune cell expression quantified in terms of mRNA expression is unlikely to be compatible with studies that measured its expression using IHC methods. In addition, TME component is different in early stage or advanced stage of NSCLC. Therefore, the role of TILs in EGFR-mutated tumors should be further investigated with a standard evaluation platform and methodology.

**Immune Cell Infiltration**

**Dendritic Cells**

Dendritic cells (DCs) are crucial antigen-presenting cells that shape T cell activation and differentiation. DCs orchestrate immune responses according to the state of maturation. Matured DCs initiate regulation of immune responses, whereas immature DCs induce immune tolerance.\textsuperscript{99} STAT3 activation mediated by interleukin (IL)-6 suppressed the maturation of DCs. Hyperactivation of STAT3 was also found in patients with EGFR-mutated NSCLC, hence induced the production of IL-6. Therefore, IL-6/gp130/STAT3 in DC is the critical signaling in mediating suppressive T cell response.\textsuperscript{100} Furthermore, activation of STAT3 also promoted the production of indoleamine 2,3-dioxygenase (IDO). IDO inhibits the activation of effector T cells by means of depletion of the essential amino acid tryptophan and promotes differentiation and activation of Tregs by means of production of kynurenine.\textsuperscript{101} In addition, IDO produced by tumor cells or DCs expanded, recruited, and activated MDSC in Treg-dependent manner.\textsuperscript{102} Nevertheless, it was reported that patients with EGFR-mutated NSCLC had a higher density of matured DCs.\textsuperscript{96,103} Although the abundant DCs were present in EGFR-mutated TME, the functions were dampened. Yu et al.\textsuperscript{104} established the EGFR-19 deletion (19DEL)-LLC mouse model to investigate the characteristics of EGFR-mutated TME. In the xenograft model, EGFR-19 deletion-expressing LLC tumors had low density of T cells and various phenotypes of DCs. They found that exosomes from EGFR-mutated LLC cells could induce anergic DCs to repress antitumor immunity in vitro instead of affecting T cell proliferation directly. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was responsible for the regulation of DC development and maintenance of DC functions. Therefore, combination of gefitinib and GM-CSF could recover T cell infiltration and enhance anti–PD-1 treatment efficacy, which needs further investigation.\textsuperscript{104}

**Tregs**

Tregs are regarded as the important suppressive immune cells in TME which contributed to tumor progression and failure of antitumor immunity.\textsuperscript{105} PD-1/PD-L1 axis played a crucial role in regulating Treg development and function.\textsuperscript{106} A previous study reported that patients with EGFR-mutated NSCLC with strongly positive PD-L1 had better efficacy than those patients with PD-L1 negative or low expression after EGFR TKI treatment with median PFS of 7.1 months; however, the results could not be compared with the satisfactory data reported in EGFR-wild type patients (mPFS of 10.3 mo in KEYNOTE-024). The possible reason might be the increased numbers of Tregs owing to the previous report that Foxp3\textsuperscript{+} T cell density was related to high PD-L1 expression in patients with EGFR-mutated NSCLC before or after EGFR TKI treatment.\textsuperscript{13,107} Amphiregulin (AREG) secreted by mast cells is the ligand for EGFR and enhances the function of Tregs by means of binding to the EGFR on Tregs and triggers the activation of AREG-induced EGFR signaling by means of EGFR/glycogen synthase kinase 3β/Foxp3 axis.\textsuperscript{108,109} Besides, EGFR mutations constantly activate JNK/cJun pathway and subsequently increase CCL22 expression, which recruit Tregs and result in higher Tregs infiltration in EGFR-mutated LUADs than in EGFR wild-type LUADs.\textsuperscript{52} Therefore, activation of EGFR pathway is critical to generate or activate Tregs. Nevertheless, evidence also revealed that the density of Foxp3\textsuperscript{+} Tregs had no substantial difference between patients with mutant and wild-type EGFR in both the tumor nest and stroma.\textsuperscript{110} The studies mentioned previously only evaluated the quantity of Treg cells, but did not further investigate the subtypes and qualitative differences in Tregs. Therefore, the detailed classification of Tregs in EGRF-mutated TME is supposed to be clarified to better characterize correlations between Tregs infiltration and EGFR mutation status.

**Tumor-Associated Macrophage**

The presence of tumor-associated macrophage (TAM) is related to resistance to immunotherapy and poor
Other Immune Cells

TME contains many other immune cells that also play an important role in exerting antitumor activity. Other T cell subsets including T helper (Th)1, Th2, Th17, and γδ T cells existed in TME that infiltrated in tumor islets or at tumor-invasive margin. The function of CD8+ T cells was supported by Th1 cells that were characterized as high production of IL-2 and IFN-γ. High density of Th1 cells was correlated with good prognosis. Th2 cells, which were characterized as production source of IL-4, IL-5, and IL-13, supported B cell function and were generally thought to promote tumor growth. Previous evidence revealed that higher level of baseline Th1 cells in peripheral blood was associated with preoperative radiological response, whereas higher Th2 cells were correlated with progressive disease in melanoma patients who were treated with ipilimumab in combination with high-dose IFN-α as neoadjuvant therapy. Nevertheless, in breast cancer, Th2 cells were found to be associated with better prognosis. EGFR tumors were reported to have less infiltrated Th2 cells. Therefore, the role of Th2 cells in EGFR-mutated tumors should be further evaluated. γδ T cells were characterized as innate immune cells and revealed potent cytotoxic ability against malignant cells. In NSCLC, the number of activated γδ T cells was associated with better clinical outcome and one study reported that EGFR-mutated tumors had fewer γδ T cells.

NK cells are crucial components in TME and orchestrate innate and adaptive immune responses by means of various cytokines and chemokines to fight against tumor. Human NK cells were often characterized as CD3-CD56+ cells, and on the basis of CD56 expression, NK cells could be subdivided as CD56dim and CD56bright cells. CD56dim NK cells had more capacity than CD56bright NK cells to produce perforin. Previous evidence revealed that ineffective NK cell activation or the lack of tumor-specific NK cells could lead to dismal results of immune-oncologic approaches. It was reported that EGFR-mutated tumors had less infiltrated CD56dim NK cells. In addition, numerous studies found that NK cells in tumor stroma were revealed as phenotypically anergic owing to the induction of transforming growth factor (TGF)-β. More research on whether the level of TGF-β in EGFR-mutated tumors could influence the function of NK cells are warranted.

MDSCs played a critical role in blocking antitumor activity by means of releasing IL-10, TGF-β, and IL-6 to suppress the function of T cells, NK cells, and DC function. Nevertheless, the infiltration of MDSC in baseline EGFR-mutated tumors is poorly studied. Jia et al. revealed that with the continuation of EGFR TKI, the numbers of MDSCs were increasing. Nevertheless, the interactions between EGFR signaling pathway and MDSC recruitment or activation remains to be characterized.

There were very few studies on the characterization of infiltration of these immune cells in EGFR-mutated tumors. The complex interactions between these cells and their communications with EGFR-mutated cells required further investigation, whether the quantity or quality of these cells in EGFR-mutated tumors influences the poor response.

Soluble Molecules

Exosome

Exosome is a small vesicle ranging 30 to 200 nm and secreted from cells to mediate signaling transduction in TME. Oncogenic EGFR could be shed from cells in the form of exosomes and interacted with other cells in TME. Previous study revealed that EGFR-containing exosomes derived from gastric cancer cells could regulate liver microenvironment to promote gastric cancer liver metastasis. In addition, exosomes containing activated EGFR could be captured by endothelial cells, in
which they elicited the EGFR-dependent response and induced the secretion of vascular endothelial growth factor (VEGF).\textsuperscript{132} It was reported that exosomes from patients with EGFR-mutated NSCLC could promote CD8\(^{+}\) T cell apoptosis which possibly impedes the efficacy of immunotherapy in these patients.\textsuperscript{133} In addition, EGFR-containing exosomes from lung cancers could induce tolerogenic DCs, which further induced the generation of tumor-specific Tregs, hence formed an immunosuppressive TME.

**Cytokines**

Cytokines, as vital regulators of immune system, modulate TME by regulating immune cell differentiation and polarization or directly contributing to tumor initiation and progression.\textsuperscript{134}

VEGF is mainly secreted by tumor or stromal cells to promote angiogenesis and recruits immunosuppressive cells.\textsuperscript{135} EGFR activation can constitutively up-regulate HIF-1\(\alpha\) in a hypoxia-independent manner and lead to VEGF gene expression.\textsuperscript{136,137} Moreover, the cross-talk between VEGF and EGFR downstream pathways leads to the elevated VEGF level in EGFR-mutated lung cancer cell\textsuperscript{138} and with the application of EGFR TKI down-regulates VEGF expression.\textsuperscript{139}

IL-6 promotes tumor growth by inhibiting apoptosis, inducing tumor angiogenesis,\textsuperscript{140} maintaining immature DCs and differentiation of MDSCs.\textsuperscript{100,141} The expression of IL-6 was correspondingly elevated in the EGFR-mutated group in both lung adenocarcinoma cell lines and mouse models.\textsuperscript{142} TGF-\(\beta\) is a crucial regulator of epithelial-mesenchymal transition (EMT), which induces abundant tumor-stromal compartments that prevent T cell infiltration.\textsuperscript{143} EGFR activation plays a role in TGF-\(\beta\)-induced enhancement of the migration and invasion ability, and the synergistic effect can be inhibited by knockdown of EGFR gene.\textsuperscript{144}

Tumor necrosis factor (TNF) plays a dual role in cancer immunity. It induces T-cell adhesion and recruits immunosuppressive cells, such as Treg and MDSC, and impairs infiltration of CD8\(^{+}\) TILs.\textsuperscript{134} EGFR activation up-regulates miR-21, which impairs the stability of TNF mRNA and decreases the TNF level.\textsuperscript{145}

As chemoattractants are responsible for leukocyte recruitment, IL-8 was associated with poor response to anti-PD-1 treatment in patients with melanoma and NSCLC.\textsuperscript{146} In EGFR-mutated lung cancer cells, IL-8 expression was elevated by means of the activation of PI3K/AKT/ERK pathway.\textsuperscript{147,148} Therefore, clarifying the role of IL-8 in patients with EGFR-mutated NSCLC helps develop further cytokine-based treatment strategy.

Despite many preclinical evidences indicated that the mutant EGFR regulates cytokines in different manners as mentioned previously, some clinical data revealed inconsistent results. Le et al.\textsuperscript{149} reported that IL-6, VEGF, and TGF-\(\beta\) were not expressed differently between EGFR-mutated and EGFR wild-type groups, but IFN-\(\gamma\) gene expression and signatures were lower in the EGFR-mutated tumors. Jacobs et al.\textsuperscript{150} reported that the serum TNF-\(\alpha\) level was elevated in patients with EGFR-mutated NSCLC (1.94 versus 1.64, \(p = 0.04\)) whereas those of TGF-\(\alpha\) and IL-6 were not significantly associated with EGFR status. Sunaga et al.\textsuperscript{148} reported that IL-8 expression in tumor specimens of patients with NSCLC with EGFR mutations was not of significant difference with those with wild-type EGFR, which contradicted to the results from tumor cell lines. The complexity of immune modulation mechanisms in TME may influence the cytokine levels and contribute to the controversies between the preclinical and clinical experimental results. Thus, more attempts are required for clarifying the precise and detailed modulation network of vital cytokines in EGFR-mutated NSCLC.

**Impact of EGFR TKI on EGFR-Mutated TME**

EGFR TKI is the standard treatment for patients with EGFR-mutated NSCLC in the first-line setting. Several studies have revealed that inhibition of EGFR and related downstream pathways resulted in up-regulation of MHC Class I molecule with more possibility of recognizable antigens and thus promote antitumor activity of CD8\(^{+}\) TILs.\textsuperscript{151-153} In addition, TMB also increased after EGFR TKI treatment resistance. Isomoto et al.\textsuperscript{107} found that the density of CD8\(^{+}\)TILs was significantly increased after EGFR TKI treatment compared with baseline. Nevertheless, high-density CD8\(^{+}\) TILs were still maintained in those with high PD-L1 expression tumors.\textsuperscript{107} To investigate the modulation of molecular biomarkers after osimertinib treatment, paired tissue biopsy was obtained and the results revealed increased CD8\(^{+}\) T cells and reduced PD-L1 expression.\textsuperscript{154} Gefitinib was reported to increase the numbers of circulating NK cells and IFN-\(\gamma\) and reduce the level of IL-6.\textsuperscript{155} Nevertheless, erlotinib was reported to impair the T-cell–mediated immune response and increase the numbers of circulating MDSCs.\textsuperscript{156,157} Of note, it was also reported that erlotinib significantly increased the T-cell–mediated cytotoxicity on lung cancer cells.\textsuperscript{158} To explain this discrepancy, our previous study analyzed the dynamic changes in TME responding to EGFR TKIs in vivo. In the early stage, EGFR TKI increased the infiltration of cytotoxic CD8\(^{+}\) T cells and DCs and decreased the numbers of Foxp3\(^{+}\) Tregs and inhibition of M2-like polarization of macrophages. Nevertheless, with the continuation of EGFR TKI, the TME became immunosuppressive with increasing...
numbers of MDSCs and elevated numbers of IL-10 and CCL2. Therefore, these findings revealed a narrow treatment window in which EGFR TKI was most beneficial to combine with PD-1/PD-L1 blockade. In addition, different EGFR TKI treatment strategies also had distinct impact on TME. Hypofractionated EGFR TKI treatment, which was defined as high dose with a low frequent treatment, was more effective in preventing tumor relapse than standard hyperfractionated EGFR TKI treatment, which was defined as low dose with daily treatment and the antitumor effect that depended on T cells involving innate immunity and adaptive immunity.

**Potential Biomarkers for ICIs in EGFR-Mutated NSCLC**

Although patients with EGFR-mutated NSCLC had poor response to immunotherapy, some cases still revealed satisfactory clinical outcome. Better understanding of these cases could help us identify the potential population who could benefit from immunotherapy.

**Types of EGFR Mutations (Sensitive or Uncommon)**

In IMpower150, survival benefit could be observed in the group of patients with EGFR-sensitizing mutations treated with atezolizumab plus bevacizumab and chemotherapy (PFS: hazard ratio [HR] = 0.41, 95% confidence interval [CI]: 0.23–0.75; overall survival [OS]: HR = 0.31, 95% CI: 0.11–0.83) than the overall cohort of patients with EGFR-mutated NSCLC (PFS: HR = 0.61, 95% CI: 0.36–1.03; OS: HR = 0.61, 0.29–1.28). In contrast, a study that included 24 patients with EGFR mutation who received nivolumab revealed that uncommon EGFR mutation was the only predictive factor for the better efficacy of nivolumab. Chen et al. noted that rare EGFR mutations were related to higher PD-L1 expression and inflamed TME with high frequency of concurrent PD-L1 expression and abundant CD8+ TIL infiltration. In 2020 American Society of Clinical Oncology (ASCO), a study investigated the long-term response (LTR) in patients treated with anti-PD1/PD-L1 therapy. LTR was defined as PR or CR lasting for more than 24 months. Only 2% of patients with EGFR-sensitizing mutations achieved LTR. Loss-of-function variants in ARID1A, PTEN, and KEAP1 were enriched in LTR compared with short-term response. These results prompted us that features predicting LTR may be distinct from those predicting initial response. IMMUNOTARGET registry study reported that patients with EGFR mutations other than alterations in exon 19 or 21 had significantly longer PFS (other mutations versus exon 19 versus exon 21: 2.8 versus 1.8 versus 2.5 mo, p < 0.001) and OS (other mutations versus exon 19 versus exon 21: 12.8 versus 4.9 versus 10.9 mo, p < 0.001). This observation could possibly attribute to decreased mutation burden in sensitive mutations reported by Dong et al. Therefore, whether sensitive or rare mutations account for better efficacy of immunotherapy should be further evaluated.

**Subtypes of EGFR Mutations (L858R or 19DEL or T790M)**

Patients with EGFR 19DEL might have improved clinical outcomes than those with EGFR L858R. Emerging data suggested that efficacy of ICIs was more favorable in patients with EGFR L858R compared with those with 19DEL. Toki et al. used multiplexed quantitative immunofluorescence to characterize the TIL population and activation status. There was no difference in the numbers of dormant TILs (high CD3 expression with low expression of Ki67 and granzyme B) between EGFR 19DEL and L858R. Nevertheless, the number of activated T cells was higher in patients with EGFR L858R (25%, 6 of 24) than those with EGFR 19DEL (9%, 2 of 22). In addition, EGFR L858R subtype tended to have high PD-L1 expression and higher TMB. A previous study revealed that patients may benefit from PD-1 inhibitors after acquiring resistance to conventional EGFR TKI treatment without T790M mutations. Those patients may characterize with high PD-L1 expression and TMB level. This was also in line with results reported in IMMUNOTARGET registry study that patients with T790M had shortest PFS compared with other EGFR-mutated subtypes.

**Shorter PFS of Initial EGFR TKI**

A study performed by Yoshida et al. found that patients with shorter response duration to previous EGFR TKI (<6 mo) were associated with longer PFS. Our group also revealed that patients with shorter TKI PFS conferred better after response to immunotherapy with the TKI PFS cutoff of 10 months. Single-cell RNA sequencing revealed that patients with shorter TKI PFS had a relatively higher proportion of CD8+ T cells and lower ratio of M2/M1 macrophage. Nevertheless, a case with common EGFR mutation and responded well to previous EGFR TKI still benefited from nivolumab for more than 2 years. Therefore, a single classification on the basis of the PFS is only the superficial phenomenon and it was hard to define the proper cutoff for TKI-PFS. Taken together, it is of crucial importance to explore the underlying mechanism for shorter TKI-PFS, especially decipher the characteristics of baseline TME in EGFR-mutated tumors before the application of initial TKI.
**High PD-L1 Expression**

Positive PD-L1 expression was significantly correlated with a longer PFS in EGFR-mutated cohort (2.8 versus 1.7 mo, \( p = 0.01 \)).\(^{25} \) The ATLANTIC study illustrated the role of PD-L1 expression in heavily pretreated patients with EGFR-mutated NSCLC. Patients with greater than or equal to 25% of tumor cells expressing PD-L1 had better clinical outcome. In addition, in the phase 1 study of gefitinib and durvalumab for advanced NSCLC harboring EGFR-sensitizing mutations revealed that patients with PD-L1 expression greater than or equal to 20% achieved better PFS than those without (15.9 versus 9.1 mo).\(^{41} \) In KEYNOTE-021 cohort E, all of the four patients with PD-L1 tumor proportion score greater than or equal to 50% produced objective response to pembrolizumab and erlotinib.\(^{43} \) Nevertheless, in another study, even patients with EGFR-mutated NSCLC who had high PD-L1 expression, the efficacy was still scarce.\(^{30} \) EGFR mutation abrogated the predictive value of PD-L1 expression. Patients with high PD-L1 expression did not get PFS or OS benefit compared with those with negative expression on the basis of the data from MSK-IMPACT.\(^{64} \) To exclude the confounding factor of IPN-\( \gamma \)-induced PD-L1 expression, a study revealed that EGFR signaling activation was associated with high PD-L1 expression with the assessment by EGFR:GRB2 PLA signaling and inactivated TILs were predominantly presented in EGFR-mutated TME. This observation potentially prompted that PD-L1 expression was the only reflection of EGFR signaling activation rather than T cell activity.\(^{97} \) Therefore, the role of PD-L1 expression in predicting response to anti-PD-1/PD-L1 inhibitors needs to be further investigated.

**Other Factors**

In KRAS-mutated tumors, three subsets of KRAS-mutated lung adenocarcinoma were identified, by co-occurring genetic events in STK11/LKB1 (KL subgroup), TP53 (KP subgroup), and CDKN2A/B inactivation with low expression of TTF1 (KC subgroup).\(^{168} \) Notably, patients from KP subgroup was most sensitive to PD-1 blockade with increasing PD-L1 expression, more T cell infiltration, and augmenting tumor immunogenicity.\(^{169,170} \) TP53-mutated tumors were accompanied with high TMB. Similarly, patients with co-occurring EGFR and TP53 mutations might benefit from immunotherapy. For instance, patients with EGFR-mutated NSCLC with TP53 co-mutation who responded to combination therapy (PD-1 blockade plus chemotherapy) had significantly better ORR than patients with TP53 wild type (62% versus 14%, \( p = 0.04 \)).\(^{36} \) In addition, Yang et al.\(^{103} \) found that EGFR-MAPK comutations had higher level of TMB and PD-L1 expression using the data from TCGA database and Cancer Proteome Atlas. Meanwhile, the TME components were similar as that in wild-type patients which indicated that patients with EGFR-MAPK comutated NSCLC might benefit from ICI treatment.\(^{103} \)

**Future Perspectives**

The status of PD-1/PD-L1 inhibitor was not well established in the first-line setting in patients with EGFR-mutated NSCLC owing to the poor response or increasing toxicity when combining with EGFR TKI. Future directions to push the immunotherapy to the front-line will attribute to combination therapy. First, combining with immunomodulatory cytokine. With the exploration of TCGA database and PANCAN, Khalil et al.\(^{95} \) reported that 10 individual genes (IL-10, BTLA, CD8A, CD39, CCR2, CSF-1R, ICOS, CD4) were associated with improved disease-free survival in patients with EGFR-mutated NSCLC. Interestingly, IL-10 is regarded as immune-suppressed genes or molecules. Nevertheless, IL-10 was also reported to have counterintuitively potent antitumor effects.\(^{171} \) Pegilodecakin (pegylated IL-10) is a long-acting IL-10 that could increase the number and functions of CD8\( ^+ \) T cells.\(^{172,173} \) Combining peg-IL-10 with PD-1 inhibitors revealed manageable toxicity profile and antitumor activity in solid tumors, which especially achieved 43% ORR in NSCLC.\(^{174} \) In 2020 WCLC, our group reported the characteristics of TME in EGFR-mutated tumors. We found that intratumoral level of IL-10 was significantly lower in EGFR-mutated tumors compared with wild-type tumors. In vitro restimulation model revealed that IL-10 up-regulated functional markers on T cells.\(^{92} \) Therefore, the role of IL-10 in treating EGFR-mutated tumors could be further evaluated. Second, combining with agents that target immune cells. As the key modulator of antigen presentation, DC could be a promising target. GM-CSF was responsible for the regulation of DC development and maintenance of DC functions. Therefore, combination of gefitinib and GM-CSF could recover T cell infiltration and enhance anti-PD-1 treatment efficacy.\(^{104} \) Furthermore, Gu et al.\(^{175} \) found that human BDCA3\( ^+ \) DCs were critical mediators of cytotoxic T lymphocyte responses and possessed strongest capacity to activate allogenic CD4\( ^+ \) T cells against EGFR-mutated lung cancer. Therefore, DC-based antitumor vaccines might have potential clinical relevance to boost antitumor immunity in EGFR-mutated tumors.\(^{176} \) Third, combining with antiangiogenesis treatment. The oncogenic EGFR pathway could lead to up-regulation of VEGF which mediated immune escape in TME. Therefore, the role of angiogenesis is crucial in patients with EGFR-mutated NSCLC.\(^{177} \) Promising results were found in
IMpower150 which revealed the role of angiogenesis in patients with EGFR-mutated NSCLC. Therefore, to push the immunotherapy, especially PD-1/PD-L1 inhibitors to the front line, antiangiogenesis could not be ignored and the underlying mechanism should be further investigated for precise therapy.

In addition to combination therapy on the basis of ICI, cancer vaccine is a promising immunotherapeutic approach that may help to overcome the limitation of conventional cancer therapeutic strategies, which can activate the immune system and induce antitumor responses by targeting tumor antigens.\textsuperscript{178,179} CIMAvax-EGF, as the worldwide first registered lung cancer vaccine, exerts the antitumor activity by stimulating the production anti-EGF Abs, which leads to a reduced concentration of serum-circulating EGF in blood and inhibits the activation of EGFR.\textsuperscript{180,181} The safety and tolerability of EGF-Vaccine was evaluated in a phase 3 clinical trial, and no severe AEs were observed. Besides, the median survival time was 10.83 months in the vaccine arm versus 8.86 months in the control arm.\textsuperscript{182} In addition, personalized peptide vaccines (PPVs) that individually select antigens for each patient are being developed for lung cancer. A phase 1b trial of personalized neoantigen therapy plus anti–PD-1 has revealed that the administration of PPV significantly enhances the ORR of advanced NSCLC.\textsuperscript{183} Nevertheless, PPVs in the patients with EGFR-mutated NSCLC were seldom investigated. The EGFR-mutant NSCLC harbors less tumor heterogeneously for they are driven by a specific oncogenic mutation. Thus, it is worth trying to establish a database of the selected neoantigen peptides of patients with EGFR-mutated NSCLC to define several shared neoantigen of this unique less heterogeneous subpopulation.

Conclusion

Immunotherapy targeting PD-1/PD-L1 pathway has revolutionized the treatment landscape of NSCLC. Nevertheless, most patients with EGFR mutation responded poorly to anti–PD-1/PD-L1 inhibitors. From this review, it could be found that EGFR-mutated NSCLC is far more complicated than we thought. Biomarkers with high predictive value in EGFR-wild type tumors, for instance, PD-L1 expression, TMB, and CD8+ TIL density, could not be completely suitable in EGFR-mutated tumors. It is also hard to leverage a single biomarker to screen the potential population in EGFR-mutated NSCLC who could benefit from immunotherapy. It is of importance to integrate multiple parameters to establish an evaluation system on the basis of multiplexed and multiomics in such population. ICI is not the lost cause in EGFR-mutated NSCLC and there are still numerous missing blanks to be filled in. We believe that rational combination strategy will put the immunotherapy to the front-line and sharpen the sword of checkpoint inhibition again. The darkness will pass, and the dawn will ultimately come.

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