Clinical outcomes of immune checkpoint blockades and the underlying immune escape mechanisms in squamous and adenocarcinoma NSCLC

Yaru Tian1,2 | Xiaoyang Zhai2 | Weiwei Yan1,2 | Hui Zhu2 | Jinming Yu2

1Department of Radiation Oncology, Shandong Cancer Hospital and Institute, Shandong University, Jinan, China
2Department of Radiation Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Science, Jinan, China

Correspondence
Hui Zhu and Jinming Yu, Department of Radiation Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Science, 440 Jiyan Road, Jinan 250117, Shandong Province, China.
Email: drzhuh@126.com (H. Z.); sdyujinming@163.com (J. Y.)

Funding information
This work was supported by National Key R&D Program of China (grant number 2018YFC1313201), The Innovation Project of Shandong Academy of Medical Sciences (2019-04), and the Academic Promotion Program of Shandong First Medical University (2019ZL002)

Abstract
Immune checkpoint blockades (ICBs) have changed the standard of care of squamous and adenocarcinoma non-small cell lung cancer (NSCLC). Whereas detailed researches regarding ICBs in the two major histological subtypes are rare. In order to uncover the clinical efficacy differences between squamous and adenocarcinoma NSCLC and better understand the underlying immune-regulatory mechanisms, we compared the survival benefits of ICBs between the two subtypes by revealing phase 3 randomized trials and attempted to uncover the immune-regulatory discrepancy. Generally, compared with nonsquamous NSCLC, squamous NSCLC benefited more from ICBs in Keynote 024, CheckMate 026, CheckMate 227 and CheckMate 017 and similar in OAK, but less in Keynote 010 and PACIFIC. We revealed that the tumor mutation burden (TMB) level, the programmed cell death ligand 1 (PD-L1) expression, tumor infiltrating lymphocytes (TILs) in the tumor microenvironment (TME), chemokines, and oncogenic driver alterations within the two subtypes may contributed to the clinical outcomes of ICBs. We prospected that the combinations of ICBs with chemotherapy, radiation therapy, and antiangiogenic therapy could be promising strategies to re-immunize the less immunogenic tumors and further enhance the efficacy of ICBs.

Keywords
adenocarcinoma NSCLC, immune checkpoint blockades, immune escape mechanisms, squamous NSCLC

1 | INTRODUCTION

Lung cancer remains the leading cause of cancer incidence and mortality in the world, with an estimated 2.1 million new cases and 18.4% of the total cancer-related deaths in 2018.1 Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers.2,3 Over the past two decades, the increasingly understanding of the biology of NSCLC has revolutionized the treatment paradigm from traditional cytotoxic chemotherapy to personalized medicine, characterized by the development of small molecule tyrosine kinase inhibitors (TKIs) and immune checkpoint blockades (ICBs), based on the genetic alterations and the programmed cell death protein 1 (PD-1) and its ligand (PD-L1).4,5 Anti-PD-1/PD-L1 therapy was an approach to “immune normalization,” which selectively restored the
tumor-induced immune deficiency in the tumor microenvironment (TME) with fewer immune-related adverse events (irAEs).6

The most common subtypes of NSCLC, squamous and adenocarcinoma NSCLC, have different origins. Basal cells in the proximal airway are considered to be the origin for squamous NSCLC, while adenocarcinoma NSCLC origins from type II pneumocytes, junction cells, and club cells of the bronchoalveolar duct.7,8 As a result, the majority of genomic alterations are distinct between squamous (e.g., cyclin dependent kinase inhibitor 2A (CDKN2A) and tumor protein p53 (TP53)) and adenocarcinoma (e.g., KRAS proto-oncogene (KRAS) and epidermal growth factor receptor (EGFR)) NSCLC.7 ICBS have dramatically altered the therapeutic landscape of advanced NSCLC. While the potential differences of immunotherapy between the two subtypes have not been fully evaluated yet. This review mainly discussed the clinical efficacy of ICBS and the dysfunctional immune microenvironment between squamous and adenocarcinoma NSCLC, and provided potential strategies to improve the clinical outcomes of immunotherapy.

2 CLINICAL OUTCOMES OF ICBS IN SQUAMOUS AND NONSQUAMOUS NSCLC.

The phase 3 randomized trials suggested that ICBS significantly improved the overall survival in patients with advanced squamous and nonsquamous NSCLC, but the clinical efficacy still varied between the two histological types.

2.1 First-line

ICBS have revolutionized the first-line treatment of advanced NSCLC. In Keynote 024,9,10 pembrolizumab significantly prolonged progression-free survival (PFS) and overall survival (OS) of patients with advanced NSCLC and PD-L1 tumor proportion score (TPS) ≥50% (Table 1). And the OS improvement was more beneficial in squamous (hazard ratio (HR), 0.35; 95% confidence interval (CI), 0.17–0.71) than nonsquamous (0.55; 95% CI, 0.39–0.76) NSCLC. Keynote 042,11 which extended the patient population to PD-L1 TPS ≥1%, also suggested that patients with TPS ≥50% benefited more from pembrolizumab than those with TPS 1–49% (HR for OS, 0.69 vs. 0.92, Table 1). Whereas nivolumab was not associated with significantly longer survival among patients with PD-L1 TPS ≥5% in CheckMate 02612 (Table 1). But for the subgroup analysis, OS was more improved in squamous compared with nonsquamous NSCLC (HR, 0.82 vs. 1.17, Table 1).

The combination of ICBS and chemotherapy has made a synergistic effect in treating advanced NSCLC. Compared with chemotherapy, the addition of pembrolizumab resulted in greatly improved OS for squamous and nonsquamous NSCLC in Keynote 40713 and Keynote 18914 (Table 1). OS improvements were similar among PD-L1 subgroups in Keynote 407, but increased with PD-L1 expression in Keynote 189 (HR, 0.59, 0.55 and 0.42 for TPS <1%, 1%–49% and ≥50%, respectively). Moreover, atezolizumab plus chemotherapy significantly prolonged PFS in squamous and nonsquamous NSCLC (Table 1). For squamous NSCLC in IMpower131,15 PFS in the high PD-L1 (PD-L1 expression of tumor cell (TC) or immune cell (IC), (TC3 or IC3)) group benefited more from the combination therapy than the low (TC1/2 or IC1/2) or negative (TC0 and IC0) groups (Table 1). Whereas for nonsquamous NSCLC in IMpower132,16 PFS was more prolonged in the high and negative groups compared with the low group (Table 1). The risk of disease progression or death also decreased with the addition of atezolizumab to bevacizumab and chemotherapy across high, low and negative PD-L1 groups in IMpower15017 (Table 1). Teff gene-signature could also predict clinical benefit of atezolizumab. CheckMate 22718 suggested that the first-line treatment of double ICBS (nivolumab plus ipilimumab) resulted in prolonged OS, independent of PD-L1 expression (Table 1). Among PD-L1 positive subgroup, squamous NSCLC had a lower risk of death than nonsquamous NSCLC.

2.2 Second-line

For the second-line setting, Keynote 01019 suggested that pembrolizumab significantly improved the objective response rate (ORR), PFS and OS among patients with advanced NSCLC and PD-L1 TPS ≥1% (Table 1). And OS favored pembrolizumab more in TPS ≥50% group than 1%–49% group (HR 0.53 vs. 0.76, Table 1). However, squamous NSCLC benefited less from immunotherapy than nonsquamous NSCLC (HR, 0.74 vs. 0.63, Table 1). Atezolizumab resulted in a relevant improvement of OS versus docetaxel regardless of PD-L1 expression or histology in OAK20 (Table 1). The OS improvements were similar between squamous and nonsquamous NSCLC (HR, 0.74 vs. 0.73, Table 1). Nivolumab was also associated with improved ORR, PFS and OS among patients with advanced squamous and nonsquamous NSCLC in CheckMate 01721 and CheckMate 05722 (Table 1). PD-L1 expression at the cutoff value of 5% and 10% could predict the efficacy of nivolumab. In CheckMate 017, the risk of death was 41% lower with nivolumab than with docetaxel in squamous NSCLC. Whereas it was only 27% lower in patients with nonsquamous NSCLC in CheckMate 057.
| Study            | N      | Histology                  | Design                      | ORR  | mPFS   | mOS   | HR for OS (95%CI)          | HR for OS (95%CI) |
|------------------|--------|---------------------------|-----------------------------|------|--------|-------|--------------------------|------------------|
| **First-line**   |        |                           |                             |      |        |       |                          |                  |
| Keynote 024      | 305    | Squamous/                 | 1. Pembro                   | 44.8%| 10.3 m | 30.0 m| 0.60 (0.41-0.89)          |                  |
| PD-L1 ≥50%       |        | Nonsquamous               | 2. Plat-based Chemo         | 27.8%| 6.0 m  | 14.2 m|                          |                  |
| Keynote 042      | 1274   | Squamous/                 | 1. Pembro                   | 27%  | 5.4 m  | 16.7 m| 0.81 (0.71-0.93)          | ≥50%, 0.69 (0.56-0.85) |
| PD-L1 ≥1%        |        | Nonsquamous               | 2. Plat-based Chemo         | 27%  | 6.5 m  | 12.1 m|                          | 1-49%, 0.92 (0.77-1.11) |
| CheckMate 026    | 423    | Squamous/                 | 1. Nivo                     | 26%  | 4.2 m  | 14.4 m| 1.02 (0.80-1.30)          | ≥50%, 0.90 (0.63-1.29) |
| PD-L1 ≥5%        |        | Nonsquamous               | 2. Plat-based Chemo         | 33%  | 5.9 m  | 13.2 m|                          |                  |
| Keynote 407      | 559    | Squamous                  | 1. Pembro+Pacl/Nab-Pacl+Carbo| 57.9%| 6.4 m  | 15.9 m| 0.64 (0.49-0.85)          | ≥50%, 0.64 (0.37-1.10) |
|                  |        |                           | 2. Placebo+Pacl/Nab-Pacl+Carbo| 38.4%| 4.8 m  | 11.3 m|                          | 1-49%, 0.57 (0.36-0.90) |
| Keynote 189      | 616    | Nonsquamous               | 1. Pembro+Pem+Plat          | 47.6%| 8.8 m  | NR    | 0.49 (0.38-0.64)          | ≥50%, 0.42 (0.26-0.68) |
|                  |        |                           | 2. Placebo+Pem+Plat         | 18.9%| 4.9 m  | 11.3 m|                          | 1-49%, 0.55 (0.34-0.90) |
| IMpower131      | 683    | Squamous                  | 1. Atezo+Nab-Pacl+Carbo     | 49%  | 6.3 m  | 14.0 m| 0.96 (0.78-1.18)          | HR for PFS       |
|                  |        |                           | 2. Nab-Pacl+Carbo           | 41%  | 5.6 m  | 13.9 m|                          | TC3 or IC3, 0.44 (0.27-0.71) |
| IMpower132      | 578    | Nonsquamous               | 1. Atezo+Pem+Plat           | 47%  | 7.6 m  | 18.1 m| 0.81 (0.64-1.03)          | HR for PFS       |
|                  |        |                           | 2. Placebo+Pem+Plat         | 32%  | 5.2 m  | 13.6 m|                          | TC1/2 or IC1/2, 0.70 (0.53-0.92) |
| IMpower150      | 800    | Nonsquamous               | 1. Atezo+Bev+Pacl+Carbo     | 63.5%| 8.3 m  | 19.2 m| 0.78 (0.64-0.96)          | HR for PFS       |
|                  |        |                           | 2. Bev+Pacl+Carbo           | 48.0%| 6.8 m  | 14.7 m|                          | TC3 or IC3, 0.39 (0.25-0.60) |
| CheckMate 227    | 1166   | Squamous/                 | 1. Nivo+ipi                 | -    | 17.1 m | -     | 0.73 (0.64-0.84)          | ≥50%, 0.70 (0.55-0.90) |
|                  |        | Nonsquamous               | 2. Chemo                    | -    | 13.9 m | -     |                          | 1-49%, 0.94 (0.75-1.18) |
| **Second-line and beyond** |        |                           |                             |      |        |       |                          | <1%, 0.62 (0.49-0.79) |
| Keynote 010      | 1034   | Squamous/                 | 1. Pembro 2 mg/kg           | 18%  | 3.9 m  | 10.4 m| 1:3                       | ≥50%, 0.53 (0.40-0.70) |
| PD-L1 ≥1%        |        | Nonsquamous               | 2. Pembro 10 mg/kg          | 18%  | 4.0 m  | 8.5 m | 2:3                       | 1-49%, 0.76 (0.60-0.96) |
|                  |        |                           | 3. Docetaxel                | 9%   |        |       | 0.61 (0.49-0.75)          |                  |

(Continues)
2.3 CONSOLIDATION

PACIFIC23,24 was a phase 3 study comparing durvalumab as consolidation therapy with placebo in patients with stage III NSCLC who did not have disease progression after ≥2 cycles of the first-line regimens and radiotherapy. PFS and OS favored durvalumab compared to chemotherapy (Table 1). The decrease of the risk of disease progression or death decreased was more among patients with PD-L1 expression ≥25% than those with PD-L1 <25% (HR, 0.41 vs. 0.59). The clinical benefit was more favorable in nonsquamous than squamous NSCLC.

These clinical trials clearly demonstrate that immunotherapy has brought about remarkable survival benefit in advanced NSCLC. PD-L1 expression is a reliable biomarker to predict the clinical efficacy of ICBS. The survival benefit was more favorable in squamous than nonsquamous type in some studies (Keynote 024, CheckMate 026, CheckMate 227, CheckMate 017) and was similar in OAK, but was less in Keynote 010 and PACIFIC. Considering the potential clinical efficacy differences between the two histologic types, we further explored the underlying immunologic mechanisms.

### TABLE 1

| Study       | N   | Histology      | Design          | ORR  | mPFS   | mOS   | HR for OS (95%CI) | HR for OS (95%CI) |
|-------------|-----|----------------|-----------------|------|--------|-------|------------------|------------------|
| OAK20       | 1225| Squamous/Nonsquamous | 1. Atezo 2. docetaxel | 14%  | 2.8 m 4.0 m | 13.8 m | 0.73 (0.62–0.87) |                      |
|             |     |                |                 | 13%  | 9.6 m  |       |                  | TC3 or IC3, 0.41 (0.27–0.64) |
|             |     |                |                 |      |        |       | 0.74 (0.58–0.93) | TC1/2/3 or IC1/2/3, 0.41 (0.27–0.64) |
|             |     |                |                 |      |        |       | 0.70 (0.56–0.87) | TC0 and IC0, 0.75 (0.59–0.96) |
| CheckMate 01721 | 272| Squamous       | 1. Nivo 2. Docetaxel | 20%  | 3.5 m 2.8 m | 9.2 m | 0.59 (0.44–0.79) | ≥10%, 0.50 (0.28–0.89) |
|             |     |                |                 | 9%   | 6.0 m  |       |                  | ≥5%, 0.53 (0.31–0.89) |
|             |     |                |                 |      |        |       | 0.60 (0.45–1.05) | ≥1%, 0.69 (0.45–1.05) |
| CheckMate 057 22 | 582| Nonsquamous   | 1. Nivo 2. Docetaxel | 19%  | 2.3 m 4.2 m | 12.2 m | 0.73 (96%;CI, 0.59–0.89) |                      |
|             |     |                |                 | 12%  | 9.4 m  |       |                  |                      |
| Consolidation PACIFIC23,24 | 709| Squamous/Nonsquamous | 1. CRT+Durva 2. CRT+Placebo | 28.5% | 17.2 m | NR       | 0.68 (99.73%;CI, 0.47–0.997) |                      |
|             |     |                |                 | 20%  | 5.6 m  |       |                  | HR for PFS ≥25%, 0.41 (0.26–0.65) |
|             |     |                |                 |      |        |       | 0.59 (0.43–0.82) | <25%, 0.59 (0.43–0.82) |

Abbreviations: Atezo, atezolizumab; Bev, bevacizumab; Carbo, carboplatin; Chemo, chemotherapy; CI, confidential interval; CRT, chemoradiotherapy; Durva, durvalumab; HR, hazard ratio; Nab-Pacl, nanoparticle albumin-bound–paclitaxel; Nivo, nivolumab; ORR, objective response rate; OS, overall survival; Pacl, paclitaxel; Pem, pemetrexed; Pembro, pembrolizumab; PFS, progression-free survival; Plat, platinum.

**Tumor mutation burden**

The overall tumor mutation burden (TMB) of NSCLC was 8.0 mutations/megabase (Mb) and TMB was significantly lower in adenocarcinoma NSCLC with 5% of the squamous tumors and 47% of adenocarcinoma tumors harboring at least five predicted neoepitopes. As a less invasive method, blood-based TMB (bTMB) was reported to have a good correlation with tissue TMB (tTMB), and could also predict tumor responses to ICIS in patients with squamous NSCLC. Most data to date is retrospective and there are several panels to determine tTMB, and studies on bTMB between squamous and nonsquamous NSCLC are rare, which need further investigations. Furthermore, studies on bTMB between squamous and nonsquamous NSCLC are rare, which need further investigations.
3.2 | PD-L1 expression

PD-L1 expression is an effective biomarker to predict the clinical efficacy of ICBs according to phase 3 randomized trials. To better understand the underlying mechanisms, we then explored PD-L1 expression in squamous and nonsquamous or adenocarcinoma NSCLC. PD-L1 expression may vary from detecting antibodies, IHC methods or expression cells (TCs or ICs). Table 2 compared PD-L1 expression from multiple clinical studies between the two histologic types. Keynote 407\textsuperscript{13} and Keynote 189\textsuperscript{14} indicated that PD-L1 expression was similar between the two subtypes. Under the Dako PD-L1 22C3 pharmDx platform, the prevalence of PD-L1 expression was higher in squamous NSCLC. Yu H et al.\textsuperscript{33} found that 46.7% and 61.5% of squamous specimens had positive PD-L1 expression on TCs and ICs. While Shinchi Y et al.\textsuperscript{34} only detected a positive PD-L1 expression rate of 26.8% in adenocarcinoma NSCLC. Two retrospective analyses also indicated a higher percentage of PD-L1 expression in squamous than adenocarcinoma NSCLC (72.3% vs. 36.9% and 34.3% vs. 4.1%, respectively).\textsuperscript{35,36} The SP142 PD-L1 IHC assay (Ventana) detected that the prevalence of PD-L1 expression ranged from 50% to 55% in squamous NSCLC,\textsuperscript{15,25,37–39} but was almost less than 40% in adenocarcinoma NSCLC.\textsuperscript{16,25,39} And the positive PD-L1 expression rate was 83% in squamous NSCLC (CheckMate 017)\textsuperscript{21} versus 78% in nonsquamous NSCLC in (CheckMate 057).\textsuperscript{22} Mazzaschi G et al.\textsuperscript{30} found that squamous NSCLC specimens exhibited a 2.5-fold higher PD-L1 value than adenocarcinoma cases. Kim S et al.\textsuperscript{40} detected that PD-L1 positivity was observed in 28.1% of adenocarcinomas. The prevalence of PD-L1 TPS ≥5% was detected to be higher in squamous than adenocarcinoma NSCLC in two retrospective studies (31% vs. 23% and 28% vs. 20%, respectively).\textsuperscript{41,42} And the overall frequency of PD-L1 expression was 56.2% and 39.9% on TCs of squamous and adenocarcinoma NSCLC, respectively.\textsuperscript{43,44} What’s more, multivariate analysis indicated that high PD-L1 expression was independently associated with squamous histology and smokers.\textsuperscript{45–48}

These studies consistently suggested that the prevalence of PD-L1 expression was significantly higher in squamous than adenocarcinoma NSCLC, which may explain the better result of ICBs in squamous NSCLC in part.

3.3 | Tumor infiltrating lymphocytes and chemokines

Tumor infiltrating lymphocytes (TILs) and chemokines play an important role in regulating immune response. Thus, we attempted to find out the immune microenvironment in the two NSCLC subtypes.

The immune cells were less functional in adenocarcinoma than squamous NSCLC. Kinoshita T et al.\textsuperscript{49} determined that the insufficiently activated infiltrating CD8+ T cells,

| Study          | PD-L1 antibody | PD-L1 expression | N  | Squamous | N  | Nonsquamous/Adeno |
|----------------|----------------|------------------|----|----------|----|-------------------|
| Keynote 407\textsuperscript{13} | 22C3/Agilent    | TC               | 559| 63.1%    |    |                   |
| Keynote 189\textsuperscript{14} | 22C3/Agilent    | TC               | 616| 63.0%    |    |                   |
| Yu H et al\textsuperscript{33}  | 22C3/Dako       | TC               | 255| 46.7%    |    |                   |
| Shinchi Y et al\textsuperscript{34} | 22C3/Dako      | TC               | 231| 26.8%    |    |                   |
| Lee SE et al\textsuperscript{35} | 22C3/Dako       | TC               | 188| 72.3%    |    |                   |
| Pan Y et al\textsuperscript{36}  | 22C3/Dako       | TC               | 108| 34.3%    |    |                   |
| IMpower131\textsuperscript{15} | SP142/Ventana   | TC/IC            | 683| 51.4%    |    |                   |
| Takada K et al\textsuperscript{37} | SP142/Ventana   | TC/IC            | 202| 52.5%    |    |                   |
| IMpower132\textsuperscript{16} | SP142/Ventana   | TC/IC            | 578| 31.3%    |    |                   |
| IMpower150\textsuperscript{17} | SP142/Ventana   | TC/IC            | 800| 51%      |    |                   |
| Chen Y et al\textsuperscript{25} | SP142/Ventana   | TC/IC            | 51 | 55%      | 136| 37%               |
| Janic U et al\textsuperscript{39} | SP142/Ventana   | TC/IC            | 25 | 52% (≥5%) | 29 | 17% (≥5%)         |
| CheckMate 017\textsuperscript{21} | clone 28–8/Dako | TC               | 272| 83%      |    |                   |
| CheckMate 057\textsuperscript{22} | clone 28–8/Dako | TC               | 582| 78%      |    |                   |
| Kim S et al\textsuperscript{40}  | E1L3N/CST       | TC               | 146| 28.1%    |    |                   |
| Parra ER et al\textsuperscript{42} | E1L3N/CST       | TC               | 108| 31% (≥5%)| 146| 23% (≥5%)         |
| Schmidt LH et al\textsuperscript{41} | E1L3N/CST       | TC               | 149| 28% (≥5%)| 125| 20% (≥5%)         |
| Yang CY et al\textsuperscript{43,44} | Polyclonal Ab/ Proteintech | TC | 105| 56.2%    | 163| 39.9%             |
immune-regulatory CD8+FOXP3+T cells and immune-dysfunctional CD8+GATA3+ T cells contributed to the immunosuppressive microenvironment in non-smokers with adenocarcinoma. Additionally, the enrichment of Foxp3+Tregs was associated with a drastic decrease of NK cells in adenocarcinoma samples and metastatic lymph nodes. In contrast, squamous carcinomas displayed less profound accumulation of Tregs.50 In elderly patients with adenocarcinomas, despite of the increased number of CD8+ T cells, the expressions of cytolytic molecule (granzyme B, perforin 1, granzyme A, granzyme M, and granulysin) were impaired, which was associated with a loss of clonal neoantigens. A number of immunosuppressive elements were upregulated, including Treg cells and co-inhibitory molecules (e.g., T cell immunoglobulin and mucin domain-containing protein-3 (TIM-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), and HERV-H LTR-associating 2 (HHLA2)).51 HHLA2, a newly discovered member of B7 family, was associated with EGFR mutation and was higher in lung adenocarcinoma compared with squamous NSCLC.52 A high FOXP3/CD4 ratio and a low number of CD20+ B cells were identified as negative prognostic factors in adenocarcinomas.53 The lack of memory B cells or increased numbers of CD20+ B cells were identified as negative prognostic factors in adenocarcinomas.54 The lack of memory B cells or increased M0 macrophages in adenocarcinoma NSCLC were correlated with the poor prognosis. Whereas T follicular helper cells in squamous NSCLC were associated with favorable prognosis.55

A significant 2.5-fold higher average PD-L1 expression, threefold increase in CD57+ cytotoxic cells and 1.5-fold increase in PD-1+ lymphocytes was detected in squamous samples compared to adenocarcinomas.55 A high level of intraepithelial CD45RO+ TILs in lymph-node metastases was an independent positive prognostic factor for PFS in squamous NSCLC, but not in adenocarcinoma NSCLC patients.56

Chemokines play an important part in regulating immune function in TME. In adenocarcinomas, bone morphogenetic protein-4 (BMP4), one of the tumor-derived regulatory programs, could augment PD-L1 expression in the mesenchymal subset of lung cancer cells.57 Adenocarcinomas had higher levels of MCP1/CCL2 and MIP-1β/CCL4 than squamous NSCLC. CCL2 and CCL4 overexpression was associated with beneficial OS and PFS in squamous NSCLC, but unfavorable OS and PFS in adenocarcinoma NSCLC.52 What's more, glycogen branching enzyme (GBE1) was also involved in the immune dysregulation in adenocarcinoma NSCLC. GBE1 blockade promoted the secretion of CCL5 and CXCL10 to recruit CD8+ T cells to the TME via the IFN-Ι/STING signaling pathway.58

3.4 | ICBs in patients with oncogenic driver mutations

Adenocarcinoma NSCLC is characterized of high prevalence of oncogenic driver mutations, with EGFR mutation rate of 27% and anaplastic lymphoma kinase (ALK) rearrangement rate of <8%.4 However, most clinical trials excluded patients with sensitizing EGFR or ALK mutations. Table 3 summarized studies evaluating clinical outcomes of ICBs in EGFR-mutant population.

Keynote 01019 indicated that the EGFR WT population significantly benefited from ICBs compared with the EGFR-mutant population (HR for OS, 0.66 vs. 0.88). The subgroup analysis of OAK20 suggested an improved survival in the EGFR WT population (HR, 0.83; 0.58–1.18), but a worse survival in the EGFR-mutant population (HR, 1.24; 0.71–2.18). The positive EGFR mutation was also a negative prognostic factor in CheckMate 05722 (HR, 1.18; 0.69–2.00). What’s more, consolidation durvalumab remarkably decreased the risk of disease progression in locally advanced NSCLC patients without sensitizing EGFR mutations (HR, 0.47; 0.36–0.60) but not in those with mutations (HR, 0.76; 0.35–1.64).23 A phase 2 study revealed that the first-line pembrolizumab lacked efficacy in PD-L1+, EGFR-mutant patients.59 In the real-world practice, EGFR mutation or ALK rearrangement was an independent negative predictor of OS in patients treated with anti-PD-1 therapy.60 A pool-analysis of four randomized control trials confirmed that patients with EGFR WT, but not EGFR mutation, could benefit from PD-L1/L1 inhibitors.28 A meta-analysis demonstrated that ICBs significantly prolonged OS in the EGFR WT subgroup (HR, 0.66; 0.58–0.76) but not the EGFR-mutant subgroup.61 What's more, Cho JH et al.62 found that the EGFR-mutant group receiving ICBs had a lower ORR than the EGFR WT group (15.8% vs. 32.9%). In addition, Hastings K et al.63 explored the heterogeneity of EGFR-mutant tumors and found that compared with 212 EGFR WT tumors, the clinical outcomes with PD-L1 blockade were worse in patients harboring EGFR exon 19 deletion, but similar in those with EGFR L858R mutation. They also demonstrated that this difference was due to a lower TMB in tumors with EGFR exon 19 deletion than those with EGFR L858R mutation. Yamada T et al.64 enrolled 27 patients with EGFR-activating mutations and confirmed a higher ORR and DCR in patients with uncommon EGFR mutations than those with common EGFR mutations (71% vs. 35.7% and 57% vs. 7%). Moreover, EGFR mutation patients without T790 M mutation were more likely to benefit from nivolumab, possibly because of a higher PD-L1 expression than those with T790 M mutation.65

In contrast to EGFR mutations and ALK rearrangement, patients with KRAS mutation seemed to achieve more benefit from ICBs. OS was significantly improved in KRAS-mutant subgroup receiving nivolumab in CheckMate 05722 (HR, 0.52; 0.29–0.95). In OAK,20 patients with KRAS mutation benefited more from atezolizumab (median OS, 17.2 m vs. 10.5 m; HR, 0.71; 0.38–1.35) than those with KRAS WT (13.8 m vs. 11.3 m; HR, 0.83; 0.58–1.18). Clinical activity of ICBs was higher in the KRAS group (ORR 26%; median PFS, 3.2 m) than the EGFR (12%; 2.1 m), BRAF (24%; 3.1 m) and
MET (16%; 3.4 m) group, and even lacked response in the ALK group. Another study elucidated that the favorable outcome of ICBs in BRAF mutants was probably due to a high PD-L1 expression. Even though a proportion of tumors with MET exon 14 mutation had PD-L1 expression, the median TMB was lower than unselected patients, and clinical efficacy is modest.

### 3.5 Immune escape mechanisms in EGFR-mutant population

Considering the lack of clinical efficacy of ICBs in patients with positive EGFR mutations, the underlying immune escape mechanisms need to be clarified.

Multiple studies have confirmed that PD-L1 expression was associated with EGFR status. Patients with EGFR mutations had decreased PD-L1 expression according to a pool-analysis of 15 public studies. This inverse correlation between EGFR mutation and PD-L1 expression was also confirmed from the analyses of The Cancer Genome Atlas (TCGA) and Guangdong Lung Cancer Institute (GLCI) cohort. Rangachari D et al. found that PD-L1 TPS ≥50% seldom overlapped with driver oncogenes. A retrospective study in Japan only detected a 9.9% (seven of 71) TPS ≥50% rate among EGFR-mutant patients. Another Japanese study revealed that 23.9% (11 of 46) patients with TPS ≥50% had positive EGFR mutations. Gainor JF et al. also indicated that ORR was significantly lower in EGFR-mutant or ALK-positive patients (3.6%) than EGFR and ALK-WT patients (23.3%). The underlying mechanisms may involve in the low rate of concurrent PD-L1 expression and CD8+ TILs within the TME. Liu SY et al. detected a lower proportion of PD-L1+/CD8+ tumors in patients with EGFR mutation.

### Table 3 Clinical outcomes of ICBs in EGFR-mutant population

| Study                | Design       | N     | Clinical outcome |
|----------------------|--------------|-------|------------------|
| Keynote 010        | 1. Pembro    | EGFR WT, 875 | HR for OS, 95%CI |
|                     | 2. Docetaxel | EGFR Mutation, 86 | 0.66; 0.55–0.80 vs. 0.88; 0.45–1.70 |
| OAK                 | 1. Atezo     | EGFR WT, 628 | HR for OS, 95%CI |
|                     | 2. docetaxel | EGFR Mutation, 85 | 0.83;0.58–1.18 vs. 1.24; 0.71–2.18 |
| CheckMate 057     | 1. Nivo      | EGFR WT, 340 | HR for OS, 95%CI |
|                     | 2. Docetaxel | EGFR Mutation, 82 | 0.66; 0.51–0.86 vs. 1.18; 0.69–2.00 |
| PACIFIC            | 1. CRT+Durva | EGFR WT, 481 | HR for PFS, 95%CI |
|                     | 2. CRT+Placebo | EGFR Mutation, 43 | 0.47; 0.36–0.60 vs. 0.76; 0.35–1.64 |
| Lisberg A et al.   | Pembro       | EGFR Mutation, 11 | 1 case had ORR |
| Phase 2 trial      |              |       |                  |
| Dong ZY et al.28   | 1. ICBs (pembro, nivo or atezo) | EGFR WT, 1990 | HR for OS, 95%CI |
| A pool-analysis    | 2. Docetaxel | EGFR Mutation, 271 | 0.67; 0.61–0.76 vs. 1.09; 0.84–1.41 |
| Lee CK et al.61    | 1. ICBs (pembro, nivo or atezo) | EGFR WT, 1362 | HR for OS, 95%CI |
| A meta-analysis    | 2. Docetaxel | EGFR Mutation, 186 | 0.66; 0.58–0.76 vs. 1.05; 0.70–1.55 |
| Cho JH et al.62    | ICBs (pembro or nivo) | EGFR WT, 140 | ORR, 32.9% vs. 15.8% |
| Retrospective      | EGFR Mutation, 38 |       |                  |
| Hastings K et al.63| ICBs        | EGFR WT, 212 | ORR, 22% vs. 7% |
| Retrospective      | EGFR 19 deletion, 80 |       | vs. 16% |
|                     | EGFR L858R, 46 |       | HR for OS, 95%CI |
|                     | 19 deletion vs. WT | 0.69, 0.493–0.965 | 0.69–2.00 |
|                     | L858R vs. WT | 0.917, 0.597–1.409 | 0.917, 0.597–1.409 |
| Yamada T et al.64  | ICBs        | EGFR Mutation, 27 | ORR, 57% vs. 35.7% |
| Retrospective      | Uncommon, 7 |       | DCR, 71% vs. 35.7% |
|                     | Common, 20 |       |                  |

Abbreviations: Atezo, atezolizumab; CI, confidential interval; CRT, chemoradiotherapy; DCR, disease control rate; Durva, durvalumab; HR, hazard ratio; Nivo, nivolumab; ORR, objective response rate; OS, overall survival; Pembro, pembrolizumab; WT, wide type.
or ALK rearrangement (5.0%, 17/342) than those with EGFR and ALK WT (14.2%, 45/316). Dong ZY et al. also discovered a lack of T-cell infiltration and shrinking proportion of CD8+ TILs in EGFR-mutant population. In addition, EGFR activation probably contributed to the uninflamed TME and participated in immunosuppression and immune escape via generation of Tregs, tolerogenic dendritic cells (DCs), and myeloid-derived suppressor cells (MDSCs). The EGF-like growth factor Amphiregulin enhanced Tregs suppressive function via the EGFR/STAT3/Foxp3 axis. Activating signal transducer and activator of transcription 3 (STAT3), a downstream signaling molecule of EGFR, inhibited DCs maturation, and promoted MDSC-mediated immunosuppression.

The immune microenvironment was consistent with the distinct immune response of EGFR- and KRAS-mutant patients. Huynh TG et al demonstrated that concurrent PD-L1 expression and abundant CD8+ TILs were observed in 25% of KRAS mutants or cases without alterations versus only 7.4% of EGFR mutants. In contrast to the low immune infiltration associated with EGFR mutations, KRAS mutations were significantly associated with T cell infiltration. Although lymphocytes were present in TME, EGFR-mutant tumors had a high frequency of inactive TILs. While TILs in KRAS mutants were almost active, inflamed with higher CD4+, CD8+, and CD20+ TILs. They also revealed that activated EGFR correlated with increased PD-L1 expression in EGFR mutants but not in EGFR WT, whereas TIL activation was associated with higher PD-L1 only in EGFR/KRAS WT. Thus, PD-L1 may reflect the constitutive oncogenic signaling in EGFR mutants rather than immune signaling in EGFR WT, which would be associated with high PD-L1 levels and TILs activation.

4 | PROSPECTIVE

Adenocarcinoma NSCLC seemed to be more immunosuppressive and benefited less from ICBs than squamous NSCLC. Strategies to improve the clinical efficacy of ICBs in adenocarcinoma is in need.

4.1 | Combination of ICBs and immunogenic drugs and radiation therapy

Compared with monotherapy, the combination of ICBs and chemotherapy has improved the clinical efficacy in patients with nonsquamous NSCLC in Keynote 189 and IMpower132. Using immunogenic drugs (oxaliplatin, cyclophosphamide et al), lung adenocarcinoma tumors that lacked T-cell infiltration and resisted current treatments in mouse models could be successfully sensitized to host antitumor T-cell immunity and further response to ICBs. Combing ICBs with chemotherapy could enhance the recognition and elimination of tumor cells by the host immune system and refine the immunosuppressive TME. Radiation therapy also had a synergistic effect with immunotherapy via enhancing MHC class I expression, activating DCs and promoting cross-presentation of tumor antigens, increasing the density of TILs, modulating the expression of immune checkpoint molecules, modulating Treg populations et al. Consolidation durvalumab after concurrent chemoradiotherapy has brought about significantly prolonged OS in phase III advanced NSCLC. In addition, the PEMBRO-RT phase 2 randomized clinical trial demonstrated that the high-dose stereotactic body radiotherapy (SBRT) on a single tumor site prior to pembrolizumab remarkably enhanced tumor response in patients with metastatic NSCLC. Thus, for those tumors uninflamed with active TILs and less immunogenic, chemotherapy or radiation therapy could be effective strategies to enhance the anti-tumor activities of ICBs.

4.2 | Combination of ICBs and antiangiogenic therapy

Angiogenesis was considered as one of the hallmarks of cancer. Vascular endothelial growth factor (VEGF), the major regulator in tumor angiogenesis, contributed to immune escape via blocking DC differentiation, inhibiting T-cell development and reducing its infiltration, inducing Tregs and MDSCs et al. In IMpower150, the addition of atezolizumab to bevacizumab and chemotherapy significantly prolonged OS in patients with advanced nonsquamous NSCLC. Based on the ALTER 0303 study, the Chinese Food and Drug Administration (CFDA) approved anlotinib, an antiangiogenesis tyrosine multikinase inhibitor, as a third-line or later therapy for advanced NSCLC. Therefore, antiangiogenic therapy can be synergistic with immunotherapy and improve the clinical efficacy of ICBs.

4.3 | Combination of ICBs and EGFR-TKIs

Multiple clinical trials are exploring the effect of the combinations of EGFR-TKIs and ALK-TKIs with ICBs, the results are immature. The phase 1/2 KEYNOTE-021 study suggested that pembrolizumab plus erlotinib did not improve ORR compared with previous monotherapy studies for patients with advanced NSCLC and sensitizing EGFR mutation. And the high incidence of treatment-related toxicities associated with these combinations made this approach more controversial.
5 | CONCLUSION

Large randomized trials have confirmed the extraordinary effects of ICBs in advanced NSCLC. Squamous NSCLC may benefit more from ICBs than adenocarcinoma NSCLC in considerations of the high TMB, high PD-L1 expression, more functional TILs in the TME and chemokines. In addition, tumors with active driver mutations, especially EGFR mutations, had more uninnflamed and immunosuppressive TME and responded less for ICBs. We prospected that chemotherapy, radiation therapy, and antiangiogenic therapy may be promising to enhance the antitumor activity of ICBs.

6 | COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTION

Jinming Yu and Hui Zhu conceived and designed the study; Yaru Tian wrote the paper; Xiaoyang Zhai and Weiwei Yan coordinated on the study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals.

DATA AVAILABILITY STATEMENT

No additional data are contained in this review.

ORCID

Hui Zhu https://orcid.org/0000-0001-9422-3886
Jinming Yu https://orcid.org/0000-0001-5933-9912

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Institute NC. Seer cancer statistics review. https://seer.cancer.gov/archive/csr/1975_2014/1975–2014
3. Society AC. Cancer facts & figures 2014. Atlanta, ga: American Cancer Society; 2014.
4. Herbst RS, Morgenstern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature. 2018;553:446–454.
5. Arbour KC, Riely GJ. Systemic therapy for locally advanced and metastatic non-small cell lung cancer: a review. JAMA. 2019;322:764–774.
6. Sanmamed MF, Chen L. A paradigm shift in cancer immunother-apy: from enhancement to normalization. Cell. 2018;175:313–326.
7. Swanton C, Govindan R. Clinical implications of genomic discoveries in lung cancer. N Engl J Med. 2016;374:1864–1873.
8. Cheung WK, Nguyen DX. Lineage factors and differentiation states in lung cancer progression. Oncogene. 2015;34:5771–5780.
9. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for pd-l1-positive non-small-cell lung cancer. N Engl J Med. 2016;375:1823–1833.
10. Reck M, Rodríguez–Abreu D, Robinson AG, et al. Updated analysis of keynote-024: Pembrolizumab versus platinum-based chemotherapy for advanced non-small-cell lung cancer with pd-l1 tumor proportion score of 50% or greater. J Clin Oncol. 2019;37:537–546.
11. Mok TSK, Wu Y-L, Kudaba I, et al. Pembrolizumab versus chemotherapy for previously untreated, pd-l1-expressing, locally advanced or metastatic non-small-cell lung cancer (keynote-042): a randomised, open-label, controlled, phase 3 trial. Lancet. 2019;393:1819–1830.
12. Carbone DP, Reck M, Paz-Ares L, et al. First-line nivolumab in stage iv or recurrent non-small-cell lung cancer. N Engl J Med. 2017;376:2415–2426.
13. Paz-Ares L, Luft A, Vicente D, et al. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. N Engl J Med. 2018;379:2040–2051.
14. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl J Med. 2018;378:2078–2092.
15. Jotte RM, Cappuzzo F, Vynnychenko I, et al. Impower131: Primary pfs and safety analysis of a randomized phase iii study of atezolizumab + carboplatin +paclitaxel or nab-paclitaxel vs carboplatin + nab-paclitaxel as 1 l therapy in advanced squamous nsclc. Journal of clinical oncology : official journal of the American Society of. Clin Oncol (R Coll Radiol). 201836(18_suppl):LBA9000.
16. Papadimitrakopoulou V, Cobo M, Bordoni R, et al. Oa05.07 im- power132: Pfs and safety results with 11 atezolizumab + carbo- platin/cisplatin + pemetrexed in stage iv non-squamous nsclc. J Thorac Oncol. 2018;13:S332–S333.
17. Soticinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous nsclc. N Engl J Med. 2018;378:2288–2301.
18. Hellmann MD, Paz-Ares L, Bernabe Caro R, et al. Nivolumab plus ipilimumab in advanced non-small-cell lung cancer. N Engl J Med. 2019;391(21):2020–2031.
19. Herbst RS, Baas P, Kim D-W, et al. Pembrolizumab versus docetaxel for previously treated, pd-l1-positive, advanced non-small-cell lung cancer (keynote-010): a randomised controlled trial. The Lancet. 2016;387:1540–1550.
20. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (oak): A phase 3, open-label, multicentre randomised controlled trial. The Lancet. 2017;389:255–265.
21. Brahmjer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med. 2015;373:123–135.
22. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015;373:1627–1639.
23. Antonia SJ, Villegas A, Daniel D, et al. Durvalumab after chemo- 
radiotherapy in stage iii non-small-cell lung cancer. N Engl J Med. 
2017;377:1919–1929.
24. Antonia SJ, Villegas A, Daniel D, et al. Overall survival with 
durvalumab after chemoradiotherapy in stage iii nsclc. N Engl J Med. 
2018;379:2342–2350.
25. Chen Y, Liu Q, Chen Z, et al. Pd-11 expression and tumor muta-
tional burden status for prediction of response to chemotherapy 
and targeted therapy in non-small cell lung cancer. J Exp Clin Cancer Res. 
2019;38:193.
26. Jiang T, Shi J, Dong Z, et al. Genomic landscape and its correla-
tions with tumor mutational burden, pd-11 expression, and 
immune cells infiltration in chinese lung squamous cell carcinoma. J 
Hematol Oncol. 2019;12:75.
27. Campbell JD, Alexandrov A, Kim J, et al. Distinct patterns of so-
matic genome alterations in lung adenocarcinomas and squamous 
cell carcinomas. Nat Genet. 2016;48:607–616.
28. Dong Z-Y, Zhang J-T, Liu S-Y, et al. Egfr mutation correlates with 
uninflamed phenotype and weak immunogenicity, causing im-
paired response to pd-1 blockade in non-small cell lung cancer. 
Oncoimmunology. 2017;6:e1356145.
29. Offin M, Rizvi H, Tenet M, et al. Tumor mutation burden and effi-
cacy of egfr-tyrosine kinase inhibitors in patients with egfr-mutant 
lung cancers. Clin Cancer Res. 2019;25:1063–1069.
30. Wang Z, Duan J, Cai S, et al. Assessment of blood tumor muta-
tional burden as a potential biomarker for immunotherapy in pa-
ients with non-small cell lung cancer with use of a next-generation 
sequencing cancer gene panel. JAMA Oncol. 2019;5:696–702.
31. Herbst RS, Giaccone G, de Marinis F, et al. Atezolizumab for first-
line treatment of pd-11-selected patients with nsclc. N Engl J Med. 
2020;383:1328–1339.
32. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor 
mutational burden as a predictor of clinical benefit in non-small 
cell lung cancer patients treated with atezolizumab. Nat Med. 
2018;24:1441–1448.
33. Yu H, Chen Z, Ballman KV, et al. Correlation of pd-11 expres-
sion with tumor mutation burden and gene signatures for prognos-
sis in early-stage squamous cell lung carcinoma. J Thorac Oncol. 
2019;14:25–36.
34. Shinchi Y, Komohara Y, Yokomizo S, et al. Accurate expression 
of pd-l1 in lung adenocarcinoma cells: A retrospective study by 
double immunohistochemistry. Cancer Sci. 2019;110:2711–2721.
35. Lee SE, Kim YJ, Sung M, et al. Association with pd-l1 expression 
in pulmonary squamous cell carcinomas. Cancer Res. 2019;12:75.
36. Pan Y, Zheng D, Li Y, et al. Unique distribution of programmed 
death ligand 1 (pd-11) expression in east asian non-small cell lung 
cancer. J Thorac Dis. 2017;9:2579–2586.
37. Takada K, Kohashi K, Shimokawa M, et al. Co-expression of idol 
and pd-11 in lung squamous cell carcinoma: Potential targets of 
novel combination therapy. Lung Cancer. 2019;128:26–32.
38. Takada K, Okamoto T, Toyokawa G, et al. The expression of pd-11 
protein as a prognostic factor in lung squamous cell carcinoma. 
Lung Cancer. 2017;104:7–15.
39. Janzic U, Kern I, Janzic A, Cavka L, Cifer T. Pd-11 expression in 
squamous-cell carcinoma and adenocarcinoma of the lung. Radiol 
Oncol. 2017;51:357–362.
40. Kim S, Koh J, Kwon D, et al. Comparative analysis of pd-11 expres-
sion between primary and metastatic pulmonary adenocarcino-
nomas. Eur J Cancer. 2017;75:141–149.
41. Schmidt LH, Kümmler A, Göricht D, et al. Pd-1 and pd-11 expres-
sion in nsclc indicate a favorable prognosis in defined subgroups. 
PLoS One. 2015;10:e0136023.
42. Parra ER, Behrens C, Rodriguez-Canales J, et al. Image analysis-based 
assessment of pd-11 and tumor-associated immune cells density sup-
ports distinct intratumoral microenvironment groups in non-small 
cell lung carcinoma patients. Clin Cancer Res. 2016;22:6278–6289.
43. Yang CY, Lin MW, Chang YL, Wu CT, Yang PC. Programmed 
cell death-ligand 1 expression is associated with a favourable im-
mune microenvironment and better overall survival in stage i pul-
monary squamous cell carcinoma. Eur J Cancer. 2016;57:91–103.
44. Yang CY, Lin MW, Chang YL, Wu CT, Yang PC. Programmed 
cell death-ligand 1 expression in surgically resected stage i pulmo-
nary adenocarcinoma and its correlation with driver mutations and 
clinical outcomes. Eur J Cancer. 2014;50:1361–1369.
45. Igawa S, Sato Y, Ryuge S, et al. Impact of pd-11 expression in patients 
with surgically resected non-small-cell lung cancer. Oncology. 
2017;92:283–290.
46. Cao L, Wang X, Li S, et al. Pd-11 is a prognostic biomarker in 
resected nsclc patients with moderate/high smoking history and 
elevated serum scca level. J Cancer. 2017;8:3251–3260.
47. Mandarano M, Bellon G, Belladonna ML, et al. Assessment of 
tils, ido-1, and pd-11 in resected non-small cell lung cancer; an 
immunohistochemical study with clinicopathological and prognostic 
implications. Virchows Arch. 2019;474:159–168.
48. Inamura K, Yokouchi Y, Kobayashi M, et al. Tumor b7–h3 (cd276) 
expression and smoking history in relation to lung adenocarcinoma 
prognosis. Lung Cancer. 2017:103:44–51.
49. Kinoshita T, Kudo-Saito C, Muramatsu R, et al. Determination of 
poor prognostic immune features of tumour microenvironment in 
non-smoking patients with lung adenocarcinoma. Eur J Cancer. 
2017;86:15–27.
50. Schneider T, Kimpfler S, Warth A, et al. Foxp3(+) regulatory t 
cells and natural killer cells distinctly infiltrate primary tumors and 
draining lymph nodes in pulmonary adenocarcinoma. J Thorac 
Oncol. 2011;6:432–438.
51. Gong Z, Jia Q, Chen J, et al. Impaired cytolytic activity and loss of 
clonal neoantigens in elderly patients with lung adenocarcinoma. J 
Thorac Oncol. 2019;14:857–866.
52. Cheng H, Janakiram M, Borczuk A, et al. Hhla2, a new immune 
checkpoint member of the b7 family, is widely expressed in human 
lung cancer and associated with egfr mutational status. Clin 
Cancer Res. 2017;23:825–832.
53. Kinoshita T, Muramatsu R, Fujita T, et al. Prognostic value of 
tumor-infiltrating lymphocytes differs depending on histological 
type and smoking habit in completely resected non-small-cell lung 
cancer. Ann Oncol. 2016;27:2117–2123.
54. Liu X, Wu S, Yang Y, Zhao M, Zhu G, Hou Z. The prognostic 
landscape of tumor-infiltrating immune cell and immunomodula-
tors in lung cancer. Biomed Pharmacother. 2017;95:55–61.
55. Mazzaschi G, Madeddu D, Falco A, et al. Low pd-1 expression 
in cytotoxic cd8+ tumor-infiltrating lymphocytes confers an im-
mune-privileged tissue microenvironment in nsclc with a prognos-
tic and predictive value. Clin Cancer Res. 2018;24:407–419.
56. Kivlaer TK, Paulsen E-E, Khanhekenari MR, et al. The presence 
of intraepithelial cd45ro+ cells in resected lymph nodes with me-
tastases from nsclc patients is an independent predictor of dis-
case-specific survival. Br J Cancer. 2016;114:1145–1151.
57. Chen L, Yi X, Goswami S, et al. Growth and metastasis of lung 
adenocarcinoma is potentiated by bmp4-mediated immunosup-
pression. Oncoimmunology. 2016;5:e1234570.
74. Rangachari D, VanderLaan PA, Shea M, et al. Correlation between classic driver oncogene mutations in egfr, alk, or ros1 and 22c3-pd-l1 expression in lung adenocarcinoma. *J Thorac Oncol*. 2017;12:403–407.

75. Yoneshima Y, Ijichi K, Anai S, et al. Pd-l1 expression and cd8+ t cells infiltration in patients with egfr-mutated and alk-rearranged lung cancer. *Lung Cancer*. 2018;125:86–92.

76. Miyawaki E, Murakami H, Takahashi T. Correlation between 22c3-pd-l1 expression and egfr mutations in japanese patients with advanced lung adenocarcinoma. *J Thorac Oncol*. 2018;13:e79–e81.

77. Gainor JF, Shaw AT, Sequist LV, et al. Egfr mutations and alk rearrangements are associated with low response rates to pd-1 pathway blockade in non-small cell lung cancer: A retrospective analysis. *Clin Cancer Res*. 2016;22:4585–4593.

78. Liu S-Y, Dong Z-Y, Wu S-P, et al. Clinical relevance of pd-l1 expression and egfr mutation on the clinical efficacy of pd-1 inhibitors in patients with pulmonary adeno- carcinoma. *J Cancer Res Clin Oncol*. 2019;145:1341–1349.

79. Takada K, Okamoto T, Shoji F, et al. Clinical significance of pd-l1 expression and cd8+ t cells infiltration in patients with egfr-mutated and alk-rearranged lung cancer. *Lung Cancer*. 2018;118:36–40.

80. Ahn B-C, Pyo K-H, Xin C-F, et al. Comprehensive analysis of the characteristics and treatment outcomes of patients with non-small cell lung cancer treated with anti-pd-1 therapy in real-world practice. *J Cancer Res Clin Oncol*. 2019;145:1613–1623.

81. Haratani K, Hayashi H, Tanaka T, et al. Tumor immune microenvironment and nivolumab efficacy in egfr mutation-positive non-small-cell lung cancer based on ct90m status after disease progression during egfr-ki treatment. *Ann Oncol*. 2017;28:1532–1539.

82. Mazieres J, Drilon A, Lusque A, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: results from the immunotarget registry. *Ann Oncol*. 2019;30(8):1321–1328.

83. Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic egfr-mutated non-small cell lung cancer—a meta-analysis. *J Thorac Oncol*. 2017;12:403–407.

84. Cho JH, Jung HA, Lee S-H, et al. Impact of egfr mutation on the clinical efficacy of pd-1 inhibitors in patients with pulmonary adeno- carcinoma. *J Thorac Oncol*. 2016;11:1869–1878.

85. Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic egfr-mutated non-small cell lung cancer—a meta-analysis. *J Thorac Oncol*. 2017;12:403–407.

86. Akamine T, Takada K, Toyokawa G, et al. Association of preoperative serum crp with pd-l1 expression in 508 patients with non-small cell lung cancer: a comprehensive analysis of systemic inflammatory markers. *Surg Oncol*. 2018;27:88–94.

87. Wu L, Du H, Li Y, Qu P, Yan C. Signal transducer and activator of transcription 3 (stat3c) promotes myeloid-derived suppressor cell expansion and immune suppression during lung tumorigenesis. *Am J Pathol*. 2011;179:2131–2141.

88. Yoshizawa S, Yasuda S, Muro K, et al. Comprehensive genomic and immunological characterization of chinese non-small cell lung cancer patients. *Nat Commun*. 2019;10:1772.

89. Zhang X-C, Wang J, Shao G-G, et al. Comprehensive genomic and immunological characterization of chinese non-small cell lung cancer patients. *Nat Commun*. 2018;9:1772.

90. Park S-J, Nakagawa T, Kitamura H, et al. Il-6 regulates in vivo dendritic cell differentiation through stat3 activation. *J Immunol*. 2004;173:3844–3854.

91. Ellis LM, Hicklin DJ. Vegf-targeted therapy: mechanisms of anti-tumor immunity. *Nat Rev Cancer*. 2008;8:579–591.

92. Wang S, Zhang Y, Wang Y, et al. Amphiregulin confers regulatory t cell suppressive function via the epidermal growth factor receptor. *Immunity*. 2013;38:275–284.

93. Zal L, van Loosdregt J, Gorlani A, et al. Amphiregulin enhances regulatory t cell suppressive function and tumor invasion via the egfr/gsk-3beta/foxp3 axis. *J Biol Chem*. 2016;291:21085–21095.

94. Takada K, Okamoto T, Shoji F, et al. Clinical significance of pd-l1 expression and cd8+ t cells infiltration in patients with egfr-mutated and alk-rearranged lung cancer. *Lung Cancer*. 2018;125:86–92.

95. Ahn B-C, Pyo K-H, Xin C-F, et al. Comprehensive analysis of the characteristics and treatment outcomes of patients with non-small cell lung cancer treated with anti-pd-1 therapy in real-world practice. *J Cancer Res Clin Oncol*. 2019;145:1613–1623.

96. Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic egfr-mutated non-small cell lung cancer—a meta-analysis. *J Thorac Oncol*. 2017;12:403–407.

97. Takada K, Okamoto T, Shoji F, et al. Clinical significance of pd-l1 expression and cd8+ t cells infiltration in patients with egfr-mutated and alk-rearranged lung cancer. *Lung Cancer*. 2018;125:86–92.

98. Yu S, Liu D, Shen B, Shi M, Feng J. Immunotherapy strategy of egfr mutant lung cancer. *Am J Cancer Res*. 2018;8:2106–2115.

99. Zal L, van Loosdregt J, Gorlani A, et al. Amphiregulin enhances regulatory t cell suppressive function via the epidermal growth factor receptor. *Immunity*. 2013;38:275–284.

100. Wang S, Zhang Y, Wang Y, et al. Amphiregulin confers regulatory t cell suppressive function and tumor invasion via the egfr/gsk-3beta/foxp3 axis. *J Biol Chem*. 2016;291:21085–21095.

101. Park S-J, Nakagawa T, Kitamura H, et al. Il-6 regulates in vivo dendritic cell differentiation through stat3 activation. *J Immunol*. 2004;173:3844–3854.

102. Wu L, Du H, Li Y, Qu P, Yan C. Signal transducer and activator of transcription 3 (stat3c) promotes myeloid-derived suppressor cell expansion and immune suppression during lung tumorigenesis. *Am J Pathol*. 2011;179:2131–2141.

103. Toki MI, Mani N, Smithy JW, et al. Immune marker profiling and programmed death ligand 1 expression across nsclc mutations. *J Thorac Oncol*. 2018;13:1884–1896.

104. Zhang X-C, Wang J, Shao G-G, et al. Comprehensive genomic and immunological characterization of chinese non-small cell lung cancer patients. *Nat Commun*. 2019;10:1772.

105. Pfirsich C, Engblom C, Rickelt S, et al. Immunogenic chemother- apy sensitizes tumors to checkpoint blockade therapy. *Immunity*. 2016;44:343–354.

106. Leonetti A, Wever B, Mazzaschi G, et al. Molecular basis and rationale for combining immune checkpoint inhibitors with che- motherapy in non-small cell lung cancer. *Drug Resist Updat*. 2019;46:100644.

107. Sharabi AB, Lim M, DeWeese TL, Drake CG. Radiation and checkpoint blockade immunotherapy: radiosensitisation and po- tential mechanisms of synergy. *Lancet Oncol*. 2015;16:e498–e509.

108. Herrera FG, Bourhis J, Coukos G. Radiotherapy combination op- tions leveraging immunity for the next oncology practice. *CA A Cancer J Clin*. 2017;67:65–85.

109. Hanahan D, Weinberg RA. Hallmarks of cancer: the next genera- tion. *Cell*. 2011;144:646–674.

110. Ellis LM, Hicklin DJ. Vegf-targeted therapy: mechanisms of anti-tumor immunity. *Nat Rev Cancer*. 2008;8:579–591.

111. Manegold C, Dingemans A-M, Gray JE, et al. The potential of combined immunotherapy and antiangiogenesis for the synergistic treatment of advanced nsclc. *J Thorac Oncol*. 2017;12:194–207.
94. Soo RA, Lim SM, Syn NL, et al. Immune checkpoint inhibitors in epidermal growth factor receptor mutant non-small cell lung cancer: current controversies and future directions. *Lung Cancer*. 2018;115:12–20.

95. Yang J-H, Gadgeel SM, Sequist LV, et al. Pembrolizumab in combination with erlotinib or gefitinib as first-line therapy for advanced nsclc with sensitizing egfr mutation. *J Thorac Oncol*. 2019;14:553–559.

96. Ahn MJ, Sun JM, Lee SH, Ahn JS, Park K. Egfr tki combination with immunotherapy in non-small cell lung cancer. *Expert Opin Drug Saf*. 2017;16:465–469.

**How to cite this article:** Tian Y, Zhai X, Yan W, Zhu H, Yu J. Clinical outcomes of immune checkpoint blockades and the underlying immune escape mechanisms in squamous and adenocarcinoma NSCLC. *Cancer Med*. 2021;10:3–14. [https://doi.org/10.1002/cam4.3590](https://doi.org/10.1002/cam4.3590)