Review

It is too Early to Say NO IMMUNOTHERAPY for EGFR-Mutant NSCLC Patients

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It is too Early to Say NO IMMUNOTHERAPY *for EGFR*-Mutant NSCLC Patients

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Abstract: EGFR tyrosine kinase inhibitors (TKIs) are the preferred initial treatment for non-small cell lung cancer (NSCLC) patients harboring *EGFR* mutations. However, remission is transient, and no further effective treatment options are available for EGFR-TKI-advanced *EGFR*-mutant NSCLCs. Immunotherapy with immune checkpoint inhibitors (ICIs) induces sustained cancer remission in a subset of NSCLCs. However, ICI therapy exhibits limited activity in most *EGFR*-mutant NSCLCs. Mechanistically, the strong oncogenic EGFR signaling in *EGFR*-mutant NSCLCs contributes to the non-inflamed tumor immune microenvironment (TIME), characterized by a limited number of $CD8^+$ T cell infiltration, a high number of regulatory $CD4^+$ T cells and a high number of inactivated infiltrated T cells. Besides, *EGFR*-mutant NSCLC patients are generally non-smokers with low levels of PD-L1 expression and tumor mutation burden. However, current understanding only partially explains why a small population of *EGFR*-mutant NSCLCs durably respond to ICI therapy, resulting in many researchers actively working in this field. This review reviews the hope seen from pre-clinical studies and clinical trials which may be adopted to improve the outcome of ICI therapy in *EGFR*-mutant NSCLCs. Besides, the underlying mechanisms leading to the inferior clinical outcome of ICI therapy in *EGFR*-mutant NSCLCs are discussed.

Keywords: simmunotherapy; EGFR mutation; non-small cell lung cancer

Introduction

Lung cancer is still the leading cause of cancer-related death worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for ~85% of all lung cancers. Up to 50% of NSCLCs in Eastern Asia and 8-16% of NSCLCs in the West have epidermal growth factor receptor (*EGFR*) mutations [2-4]. *EGFR* L858R missense mutation and in-frame exon 19 deletions (19del) are the most common mutations found in *EGFR*-mutant NSCLCs, accounting for ~85% of all *EGFR*-activating mutations [3-6]. NSCLCs with *EGFR* L858R or 19del are sensitive to initial EGFR tyrosine kinase inhibitor (TKI) therapy [7, 8]. Relative to traditional chemotherapy, EGFR-TKI therapy significantly extends progression-free survival (PFS), overall survival (OS), and improved quality of life due to relatively low toxicity for NSCLC patients harboring sensitive *EGFR* mutations [9-18]. EGFR-TKI therapy is the first-line treatment for NSCLC patients with sensitive *EGFR* mutations in the National Comprehensive Cancer Network (NCCN) guidelines [19]. However, the remission is still transient for only nine months to around two years [9-16, 20]. With the universal application of EGFR TKIs to treat the *EGFR*-mutant NSCLCs, EGFR-TKI resistance represents an unmet clinical problem. So far, no further effective treatment options have been approved after progression to EGFR TKIs in *EGFR*-mutant NSCLCs, most of whom need chemotherapy-consisting therapy. However, the clinical benefit of the chemotherapy-consisting therapy is modest, with a high potential for toxicity.

Other than targeted therapies, immunotherapy with immune checkpoint inhibitors (ICIs), represented by anti-programmed cell death-(ligand) 1 [anti-PD-(L)1] therapy, has demonstrated improved survival rates, long-term survival benefits, and favorable safety profile over chemotherapy in a subset of advanced NSCLCs [21-27], leading 6 ICIs (ipilimumab, nivolumab, pembrolizumab, atezolizumab, avelumab, and durvalumab) approved by FDA as first- or second-line treatments for advanced NSCLCs but not suggested for patients with sensitive *EGFR* mutations [28]. Relative to NSCLCs with *EGFR* wild-type, ICI therapy fails to improve survival benefits in most *EGFR*-mutant

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NSCLCs [29-34]. Clinical benefit from ICI therapy is associated with inflamed tumors with infiltrated immune cells, high tumor mutation burden (TMBs), and high expression of PD-L1 on tumor cells [35]. However, the oncogenic EGFR signaling in lung cancer with *EGFR* mutations contributes to a non-inflamed tumor immune microenvironment (TIME) characterized by limited infiltration of $CD8^+$ T cells [36-38], a high number of infiltrations of regulatory $CD4^+$ T cells [38], and a high number of inactivated infiltrated T cells [39]. Besides, *EGFR*-mutant NSCLCs are generally non-smokers [34] with low PD-L1 expression [36, 37, 39] and low tumor mutation burden [36, 37]. The mechanisms mediating durable effects in a small proportion of *EGFR*-mutant NSCLCs are worth further investigation [40].

Hope:

1. The Efficacy of Osimertinib for EGFR-Mutant NSCLCs is Independent of PD-L1 Levels

Although osimertinib treatment decreased PD-L1 expression on *EGFR*-mutant NSCLC cell lines by reducing PD-L1 at mRNA level and promoting PD-L1 degradation by proteasomes [41], the median PFS (mPFS) of osimertinib was similar in PD-L1 negative patients (TC<1%) (18.9 months) and in PD-L1 positive patients (TC≥1%) (18.4 months) [42], suggesting the osimertinib efficacy was independent of PD-L1 status in *EGFR*-mutant NSCLCs.

2. Subtypes of EGFR Alterations May be Sensitive to ICI Therapy

ICI efficacy varies in subtypes of *EGFR* alterations in *EGFR*-mutant NSCLCs. *EGFR* wild-type and L858R NSCLCs had a similar outcome, while *EGFR* 19del NSCLCs had a worse outcome when treated with ICI therapy [43]. One of the possible explanations was that *EGFR* L858R-mutant tumors were more likely to have significantly increased $CD8^+$ T cell infiltration than *EGFR* 19del tumors [39]. The findings were also supported by other groups that ICI therapy alone or in combination exhibited improved survival outcomes in the *EGFR* L858R group compared with that in the *EGFR* 19del group [44-47]. Besides, *EGFR* T790M-negative patients were more likely to benefit from ICI therapy than *EGFR* T790M-positive patients [48, 49], as the *EGFR* T790M-negative tumors had higher PD-L1 expression than the *EGFR* T790M-positive tumors [48, 50]. Other than the common mutations, 10-20 % of *EGFR*-mutant NSCLCs harbor non-*EGFR* L858R or 19del mutations, known as uncommon mutations, such as S768I and G719X [5, 37, 51, 52]. Relative to common *EGFR* mutations, uncommon *EGFR*-mutant tumors were likely to express high PD-L1 (≥50%), high TMB, and high infiltration of $CD8^+$ T cells [37, 49, 52, 53]. Further, 36.7% uncommon *EGFR*-mutant NSCLCs with dual high PD-L1 expression and abundant $CD8^+$ T cell infiltration showed a favorable response to ICI therapy [49, 53].

3. Heavily Pre-Treated EGFR-Mutant NSCLCs are Likely to Respond to ICI Therapy Relative to Treatment-Naïve EGFR-Mutant NSCLCs

EGFR-TKI treatment results in dynamic changes in host immunity. EGFR-TKI-advanced *EGFR*-mutant NSCLC patients trended to have concurrent high levels of PD-L1 expression and high number of $CD8^+$ T cell infiltration [34]. It was further found that the proportion of *EGFR*-mutant NSCLCs with high PD-L1 (≥50%) was increased from 14% to 28% after EGFR-TKI treatment [54]. Further, heavily pre-treated EGFR-TKI-advanced *EGFR*-mutant NSCLCs with high expression of PD-L1 (≥25% of tumor cells) had a better median OS (mOS) (13.3 vs. 9.9 months) than that with low expression of PD-L1 (<25% of tumor cells) after ICI therapy in a Phase II ATLANTIC study [29, 55]. It was also found that 2 of 5 EGFR-TKI-advanced *EGFR*-mutant NSCLCs with increased PD-L1 after EGFR blockade showed a durable response to subsequent ICI therapy [54]. Overall, EGFR-TKI-advanced *EGFR*-mutant NSCLCs may be the groups responding to ICI therapy. However, ICI therapy immediately followed by EGFR TKI therapy may cause immune-related adverse effects (irAEs), as discussed below.

4. IrAEs Observed During ICI Treatment Are Associated with the Efficacy

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Even though irAEs are not necessary for treatment benefit, a growing body of literature supports that the occurrence of irAEs during ICI therapy is associated with improved treatment efficacy. A retrospective analysis proved that the 270 metastatic NSCLCs who experienced irAEs after ICI therapy had improved PFS, overall response rate (ORR), disease control rate (DCR), and OS relative to those who did not experience irAEs (PFS: 5.2 vs. 1.97 months; ORR: 22.9% vs. 5.7%; DCR: 76% vs. 58%; OS: not reached vs. 8.21 months) [56]. Similarly, secondary analysis of the Phase I CA209-003 clinical trial conducted at 13 US medical centers led to findings that the mOS was significantly longer among patients with irAEs of any grade (19.8 months; 95% CI, 13.8-26.9 months) or grade 3 or more (20.3 months; 95% CI, 12.5-44.9 months) compared with those without treatment-related AEs (5.8 months; 95% CI, 4.6-7.8 months) (p<0.001 for both comparisons based on hazard ratios) [21]. Another retrospective study found that around 43.6% of 195 advanced NSCLCs treated with ICI therapy experienced irAEs. Significantly longer mPFS (5.7 s. 2.0 months), PFS (8.5 vs. 4.6 months), mOS (17.8 vs. 4.0 months), and OS (26.8 vs. 11.9 months) relative to those who did not experience irAEs was observed [57]. Besides the retrospective studies, forty-three advanced NSCLCs were enrolled in a prospective study to evaluate the association between clinical outcomes and irAEs after ICI therapy. Consistently, earlier irAEs were associated with better objective response and disease control rates than those without irAEs after ICI therapy (37% vs. 17% and 74% vs. 29%, respectively) [58]. Another prospective study with 76 advanced NSCLCs also supported that the mPFS was significantly longer when the NSCLCs experienced irAEs within two weeks of beginning ICI than those who did not (5.0 vs. 2.0 months) [59]. However, it is difficult to predict the treatment-related toxicity.

5. Positive Clinical Studies Support the Use of ICI Therapy in EGFR-Mutant NSCLCs

Even though the ORR for *KRAS*-mutant NSCLCs to ICI therapy is around 20%, 7% of *EGFR*-mutant NSCLCs respond to single-agent ICI therapy [40]. A female NSCLC patient harboring *EGFR* L858R mutation treated with ICI therapy resulted in a prolonged PFS (~23 months) [60]. To achieve further improved outcomes, anti-PD-1/PD-L1 antibody-based ICI therapy, combined with other checkpoint inhibitors or platinum-based chemotherapy, has been widely applied in advanced lung cancers [28, 61]. Yang et al. observed that chemo-immunotherapy was better than immunotherapy alone for EGFR-TKI advanced *EGFR*-mutant NSCLCs (mPFS: 3.42 vs. 1.58 months, p=0.027) [62]. A retrospective study of 122 EGFR-TKI-advanced *EGFR*-mutant NSCLC patients, especially with *EGFR* L858R mutation, revealed better mPFS (5.0 vs. 2.2 months) and mOS (14.4 vs. 7 months) in ICI-based combination therapy than that in ICI alone [45]. Further, front-line ICI therapy reached better survival benefits than later-line ICI therapy [45]. In another retrospective study, chemo-immunotherapy also achieved better outcomes than ICI therapy alone of mPFS (4.3 vs. 1.5 months), mOS (14.92 vs. 7.41 months) and ORR (23.1% vs. 3.1%) in the EGFR-TKI-advanced *EGFR*-mutant NSCLCs especially with *EGFR* L858R mutation, respectively [63]. Consistent with the findings, patients with *EGFR* L858R exhibited a trend of longer mPFS (7.6 vs. 5.4 months) and mOS (23.5 vs. 18.0 months) versus patients with *EGFR* 19del receiving chemo-immunotherapy [46].

VEGF is not only associated with NSCLC progression, recurrence, and metastasis; VEGF, complimentarily with EGFR pathways, promotes an immunosuppressive TIME [64], and anti-angiogenic therapy can reprogram the TIME from immunosuppressive to immune-supportive [65, 66]. Currently, anti-angiogenic drugs plus chemotherapy are the most common regimen for EGFR-TKI-advanced NSCLCs [67]. Chemo-immunotherapy combinations achieved a higher objective response rate (ORR) relative to the chemo-anti-angiogenesis combination (29.5% vs. 13%) [67]. Longer PFS was also associated with previous anti-angiogenic drug applications in patients receiving chemo-immunotherapy [62]. Chemo-immuno-anti-angiogenesis combination was superior to either chemo-immunotherapy or chemo-anti-angiogenesis combination on mPFS (10.2, 6.9, 6.9 months), and the mOS (29.4, 19.0, 18.1 months) in the Phase III IMpower150 study in a subgroup of EGFR-TKI-advanced *EGFR*-mutant NSCLCs [68], suggesting that the combination of ICI, anti-angiogenesis, and chemotherapy may be a novel therapy for EGFR-TKI-advanced *EGFR*-mutant NSCLCs. Chemo-immuno-anti-angiogenesis combination therapy correlated with the highest rate of grade 3/4 treatment-related AEs compared to chemo-immunotherapy and chemo-anti-angiogenesis (66.7%,

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42.4%, and 13.6%) [68]. Like the IMpower150 clinical trial, 40 EGFR-TKI-advanced *EGFR*-mutant NSCLC patients received chemo-immuno-anti-angiogenesis therapy enrolled in a Phase II clinical trial, and the patients achieved impressive mPFS (9.4 months), one-year OS (72.5%), and ORR (62.5%) with only 37.5% reported irAEs [69]. These positive clinical trials may identify novel biomarkers that predict responders to ICI therapy alone or in combination for EGFR-TKI-advanced *EGFR*-mutant NSCLCs.

Challenges:

1. PD-L1 is a Debating exclusive Predictive and Prognostic Biomarker for ICI Therapy in EGFR-Mutant NSCLCs

Expression of PD-1 on activated T cells, B cells, and natural killer (NK) cells blunts the immune response through interaction with its major ligand PD-L1, expressed on tumor cells and infiltrating immune cells [70-72]. Disruption of the PD-1/PD-L1 interaction reactivates the anti-tumor T cell-mediated cell cytotoxicity. However, researchers do not consistently agree on PD-L1 expression levels in *EGFR*-mutant NSCLCs. In earlier studies, activation of the PI3K-AKT, MAPK-ERK, or JAK-STAT3 pathway by aberrant oncogenic EGFR signaling upregulated PD-L1 expression in *EGFR*-mutant NSCLC cell lines [73-77], and EGFR TKI treatment decreased PD-L1 expression [73, 74]. Some studies reported no correlation between PD-L1 expression and *EGFR* mutation status [78, 79]. In more recent studies with clinical samples, the *EGFR*-mutant NSCLC group expressed significantly lower PD-L1 than the *EGFR* wild-type NSCLC group [80-84]{Ji, 2016 #18530}{Dong, 2017 #17138}{Li, 2017 #18540}{Takada, 2018 #18462}{Lee, 2019 #18499}. A pooled analysis of 15 public studies further suggested that *EGFR*-mutant NSCLCs have a decreased PD-L1 expression [36]. PD-L1 expression was more accentuated at portions with higher PD-L1 expression in *EGFR*-mutant versus *EGFR* wild-type group (51% vs. 68% at TC≥1%, 8% vs. 35% at TC≥25% and 5% vs. 28% at TC≥50%) [42]. Furthermore, EGFR blockade upregulated PD-L1 expression in *EGFR*-mutant NSCLCs [34, 54, 85], supporting that EGFR-TKI-advanced EGFR-mutant NSCLC patients probably benefit from ICI therapy as discussed above.

PD-L1 was a predictive and prognostic biomarker for response to ICI therapy in NSCLCs [25]. Several clinical trials reported the association between PD-L1 expression and clinical outcomes in NSCLC patients [25, 33, 86]. However, PD-L1 levels do not consistently indicate ICI response and correlate with prognosis, especially for *EGFR*-mutant NSCLCs [39]. ICI therapy did not show clinical benefit in TKI naïve *EGFR*-mutant NSCLCs, even with high PD-L1 expression. A Phase II trial, despite 73% of enrolled NSCLC patients with more than 50% PD-L1 expression, mostly treatment-naïve with sensitive *EGFR* mutations, was discontinued after 11 patients due to lack of efficacy PD-L1 [33, 86]. Patients with low or even undetectable PD-L1 expression also had improved survival with ICI therapy versus chemotherapy [25]. Cross-comparison is sometimes challenging due to various immunohistochemical (IHC) assays with different scoring systems and cutoff values [87, 88], making a universal assay for assessing PD-L1 expression with appropriate cutoff points important.

Besides, the disconnection might be because PD-L1 expression could not reflect the underlying T cell activity in *EGFR*-mutant NSCLCs in earlier studies [39]. PD-L1 is also highly expressed in circulating immune cells, such as dendritic cells and myeloid-derived immune suppressor cells [89, 90]. In contrast, most clinical trials only assess tumoral PD-L1 expression when investigating ICI efficacy. Assessing PD-L1 expression in tumor cells and tumor-infiltrating immune cells in both POPLAR and OAK trials led to the finding that higher PD-L1 levels in both tumor cells and tumor-infiltrating immune cells were associated with improved patient survival after ICI therapy [25, 91]. Patients with more than 30% of PD-L1^{+}CD11b^{+} myeloid cells before ICI therapy exhibited a 50% superior response rate [92]. Furthermore, PD-L2, another ligand identified for PD-1 T cell receptor (TCR) [93], could compete with PD-L1 with 2-6 fold higher affinity to PD-1 [94]. EGFR signaling could also regulate PD-L2 expression in NSCLCs [95].

2. TMB is Low in EGFR-Mutant NSCLCs

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TMB is the total number of gene alterations, including substitutions, insertions, and deletions, correlated with higher levels of neoantigens. NSCLC patients with high TMB respond better to ICI therapy [96]. However, *EGFR* mutations are associated with decreased tumor mutation burden compared with tumors with *EGFR* wild-type [36, 37, 97]. Lung tumors with *EGFR* 19del harbored an even lower tumor mutation burden than *EGFR* L858R lung tumors [43, 97]. Older people have increased TMB [98]. *EGFR* 19del was commonly found in the young, while *EGFR* L858R predominated in the elderly [5]. In contrast to NSCLCs harboring common *EGFR* mutations, patients with uncommon *EGFR* mutations, especially the G719X mutation, showed the highest TMB (7.5 mutations/Mb), followed by *EGFR* exon 20 ins (4.6 mutations/Mb), *EGFR* T790M (4.05 mutations/Mb), *EGFR* L858R (3.4 mutations/Mb), and *EGFR* 19del (3.1 mutations/Mb) ($p<0.05$) [37]. Similar to PD-L1, high TMB has been detected in responders and non-responders receiving ICI therapy [99], indicating TMB is not the only determinant for ICI response.

3. EGFR-Mutant NSCLCs are Non-Smokers, Generally

Tobacco is a known risk factor for lung cancer. However, Smokers are more likely to benefit from ICI therapy [100-102]. Although *EGFR* mutations are more enriched in never-smokers, *EGFR* mutations in NSCLCs are also found in ever-smokers [6, 103-105]. Compared with common *EGFR* mutations, uncommon *EGFR* mutations were significantly associated with smoking [106]. PD-L1 positivity was also associated with smoking history [80-82]. These findings further support the above discussed findings that the NSCLCs with uncommon *EGFR* mutations are more likely to have a favorable response to ICI therapy than NSCLCs with common *EGFR* mutations [49, 53].

4. EGFR-Mutant NSCLCs Have a Lymphocyte-Depleted TIME

The crosstalk between cancer cells and TIME has become a hot research topic with the rapid development of immunotherapy in cancer, including lung cancer. Based on the presence or absence of tumor-infiltrating lymphocytes (TILs), there are four different types of TIME in tumors: type I: $TIL^{+}PD\text{-}L1^{+}$; type II: $TIL^{-}PD\text{-}L1^{-}$; type III: $TIL^{-}PD\text{-}L1^{+}$; type IV: $TIL^{+}PD\text{-}L1^{-}$, and only type I tumors with lymphocyte infiltration and PD-L1 expression can respond to ICI therapy [107]. To better reflect the complex relationship of the tumor, host, and environmental factors, tumors have also been classified into the following types: the immune-dessert tumor, the immune-excluded tumor, and the inflamed tumor [108]. The immune-dessert and immune-excluded tumors are naturally resistant to ICI therapy. *EGFR*-mutant tumors have a "lymphocyte depletion" phenotype [34, 36, 38, 109, 110], characterized by a pronounced lack of the infiltration of $CD8^{+}$ T cells [34, 36, 38, 111], suggesting an immunosuppressive TIME. Single-cell RNA sequencing supported the findings that fewer $CD8^{+}$ T cells and more macrophages were in the TIME when tumors were resistant to EGFR-TKI treatment than when EGFR TKIs were effective [112]. Additionally, *EGFR*-mutant NSCLC tumors had markedly less crosstalk between T cells and other cell types via the PD-1/PD-L1 pathway than *EGFR*-negative NSCLCs [113].

Regulatory T cells (Tregs), especially the Forkhead box P3 $(Foxp3)^{+}$, play essential roles in immune suppression [114]. *EGFR*-mutant NSCLC tumors showed high infiltration of $Foxp3^{+}CD4^{+}$ regulatory T cells [38, 115, 116]. Retrospective immunohistochemistry analysis of 164 *EGFR*-mutant and 159 *EGFR* wild-type tumors revealed that the expression of CD3, CD4, and Foxp3 was significantly higher in *EGFR*-mutant NSCLC tumors than that in the *EGFR* wild-type tumors [117]. EGFR blockade increased intratumor $CD8^{+}$ T cells and decreased Tregs infiltration in the TIME [38, 118-121]. Similar as Tregs, myeloid-derived suppressor cells (MDSCs), known to suppress immune response [122-124], were also found to be elevated in *EGFR*-mutant NSCLC tumors [121].

Tumors without detectable EGFR expression responded to EGFR inhibition [125], implicating that EGFR blocking blockade potentially influenced the tumor-specific immune responses. Deficient *Egfr* in murine myeloid cells decreased carcinogenesis, suggesting a tumor-promoting function by myeloid cell-intrinsic EGFR signaling [126]. Macrophages in the TIME express EGFR [126, 127]. EGF secreted by tumor cells promoted M2 polarization of tumor-associated macrophages (TAMs), associated with suppression of cytotoxic T cell function [128]. In contrast to M2-type TAMs, higher

infiltrated M1-type TAMs found in NSCLC with uncommon *EGFR* mutations (G719X and exon 20s) correlated with longer PFS than common *EGFR* mutations [37].

EGFR-mutant NSCLC cell lines significantly downregulated MHC class I molecule expression compared with the *EGFR* wild-type NSCLC cell lines in response to IFNγ [129]. PI3K-AKT and MAPK pathways, the primary downstream signaling pathways of EGFR {Yan, 2018 #7245}{Yan, 2022 #15218}, also suppressed MHC class I molecule expression [129-133], suggesting the downregulation of MHC class I molecules in *EGFR*-mutant NSCLCs is through abnormal EGFR signaling. Indeed, EGFR inhibition augmented the expression of MHC class I molecules [134-136].

5. EGFR-Mutant NSCLCs Respond Poorly to ICI Therapy Alone or in Combination

Unlike those without driver mutations, *EGFR*-mutant NSCLCs generally respond poorly to ICI therapy [30, 34]. Subgroup analysis of the patients with activating *EGFR* mutations in the first Phase III clinical trial of CheckMate-057 revealed no PFS and OS benefit from ICI treatment [23]. A retrospective analysis revealed that *EGFR* mutations were associated with low clinical response to ICI blockade in NSCLCs [34]. Subgroup analysis of the data from the Phase III clinical trial of KEYNOTE-010 also indicated no improved OS benefit from ICI treatment in *EGFR*-mutant NSCLCs [27]. Similarly, *EGFR*-mutant NSCLCs failed to achieve prolonged OS from ICI vs. chemotherapy in Phase III clinical trial OAK [25]. It was further confirmed in a pooled analysis from 3 clinical trials (CheckMate-057, KEYNOTE-010, and POPLAR) that the *EGFR* wild-type but not the *EGFR*-mutant NSCLCs had prolonged OS [31]. Combining data from 5 trials (CheckMate-017, CheckMate-057, KEYNOTE-010, OAK, and POPLAR), Lee et al. additionally confirmed no prolonged OS in *EGFR*-mutant NSCLCs receiving ICIs relative to chemotherapy [32]. In a Phase II trial, treatment of EGFR-TKI naïve *EGFR*-mutant patients with ICI was halted due to lack of efficacy and two deaths within six months of enrollment, 1 of which was from pneumonitis [33]. In the study of WJOG8515L, nivolumab was inferior to the chemotherapy treatment in EGFR-TKI-advanced *EGFR*-mutant NSCLCs without T790M mutation with worse mPFS (1.7 vs. 5.6 months) and ORR (9.6% vs. 36%) [137]. In a retrospective study, 58 *EGFR*-mutant NSCLC patients who responded to prior EGFR TKIs for more than ten months displayed significantly shorter PFS of ICIs compared to those responding to prior EGFR TKIs for less than ten months (1.6 vs. 1.9 months, p=0.009) [138]. More recently, ICI was unfavorable versus chemotherapy in EGFR-TKI-advanced NSCLCs harboring a secondary T790M mutation with worse mPFS (1.7 vs. 5.6 months) and response rate (9.6% vs. 36.0%) and similar OS (20.7 vs. 19.9 months) in Phase II WJOG8515L clinical trial [137]. Adding ICIs to chemotherapy was associated with worse survival than platinum doublet chemotherapy alone in osimertinib-advanced *EGFR*-mutant NSCLCs [139]. Phase III clinical trial IMpower130 also found no benefit in the *EGFR*-mutant subgroup treated with ICIs and chemotherapy combined versus chemotherapy alone [140]. Based on all these negative findings, the National Comprehensive Cancer Network (NCCN) clinical practice guidelines of NSCLC (version 4, 2021) did not recommend immunotherapy for treating *EGFR*-mutant NSCLCs.

6. Safety Concerns and Lower Clinical Outcomes Regarding EGFR TKI and ICI Combined for the Treatment of EGFR-Mutant NSCLCs

Compared to chemotherapy, ICI therapy correlates fewer adverse reactions. However, ICI therapy was associated with immune-related adverse events (irAEs) caused probably due to the disruption of immunologic homeostasis [141]. There is growing concern that a combination of EGFR TKIs, especially osimertinib and gefitinib, and ICI therapy may be associated with an increased risk of toxicity. Five of seven untreated stage IIIB/IV *EGFR*-mutant NSCLC patients (71.4%) treated with ICI plus gefitinib had grade 3/4 liver toxicity, leading to permanent treatment discontinuation in four patients in the Phase 1/2 KEYNOTE-021 clinical trial [142]. A lung adenocarcinoma patient bearing *EGFR* 19del treated with osimertinib following ICI therapy had Stevens–Johnson syndrome and hepatotoxicity [143]. In a Phase Ib clinical trial of TATTON, 38.5-60% of EGFR-TKI-advanced *EGFR*-mutant NSCLCs experienced grade 3/4 irAEs, and 30-40% discontinued to osimertinib and ICI combined therapy due to irAEs [144]. Concurrent osimertinib and ICI therapy were associated with

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an increased incidence of irAEs, leading to early termination of a Phase III CAURAL recruitment [145]. Interstitial lung disease (ILD) is a severe adverse response to EGFR TKIs [146]. Osimertinib treatment immediately followed prior ICI therapy, which also caused a high incidence of ILD [147, 148]. A meta-analysis of eight studies further concluded that a higher chance of irAEs was observed in EGFR-TKI plus ICI therapy in EGFR-TKI-advanced *EGFR*-mutant NSCLCs [149].

Other than toxicity concerns, lower clinical outcomes have also been observed in *EGFR*-mutant NSCLCs treated with EGFR TKI and ICI combined than those treated with EGFR TKI alone. The Phase Ib clinical trial of TATTON showed that osimertinib and ICI combination therapy only achieved 43% of ORR in the EGFR-TKI-advanced *EGFR*-mutant NSCLCs [144]. Besides, the ORR in gefitinib plus ICI therapy (14.3%) was much worse than the 55% of RR in gefitinib alone for *EGFR*-mutant NSCLC patients treated with gefitinib alone [150]. In contrast to gefitinib plus ICI therapy, the untreated stage IIIB/IV *EGFR*-mutant NSCLCs tolerated erlotinib plus ICI therapy and better ORR (41.7%) in the KEYNOTE-021 [142]. The RR was much lower than ~70% when using erlotinib alone as a first-line treatment in clinical trials [151, 152]. Decreased ORR was also observed in the osimertinib plus ICI group versus osimertinib alone in the Phase III CAURAL recruitment [145].

7. Hyper-Progressive Disease (HPD)

Hyper-progressive disease (HPD), characterized by unexpected fast tumor enlargement both at rate and volume and early fatality of patients [153, 154], was observed in 13.8% of NSCLC patients during treatment with ICIs in a retrospective study [155]. In contrast, around 20% of *EGFR*-mutant NSCLCs showed risk of HPD after ICI therapy [153].

Conclusions

So far, it has been agreed that ICI-based immunotherapy is ineffective for treatment-naïve *EGFR*-mutant NSCLC patients. Whether EGFR-TKI-advanced *EGFR*-mutant NSCLC patients may benefit from the ICI-based therapy is worth investigating, considering no further approved effective treatment for this population. irAEs, symptoms that oncologists try to avoid during the immunotherapy application, will be an unmet roadblock during the evaluation. However, irAEs may not always be bad for the patients, as it may indicate that the immune system in the body is awakened. However, it is hard to predict irAEs, and some strong irAEs, if not controlled, may be deadly. A better understanding of what types of irAEs may correlate with efficacy and when the correlated irAEs happen may be helpful for future clinical monitoring during immunotherapy application. Other than irAEs, many other questions remain unsolved. Who may benefit: PD-L1 TPS>50%, smoking history, high tumor mutation burden, high $CD8^+$ T cells, and specific subtypes of *EGFR* mutations? Besides, the combination of ICI-based immunotherapy and several other treatment modalities is still under active investigation. Furthermore, immunotherapy is not limited to anti-PD-1/PD-L1 monoclonal antibodies and could go beyond them.

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