Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-21-572

Reviewer A

Authors reviewed TIME in NSCLC after EGFR-TKI therapy and its role in TKI resistance.

1. EGFR-TKI resistance is related to M2-type TAMs, decreased numbers of DCs and their dysfunction.

2. A combination of EGFR-TKIs and NK cell adoptive immunotherapy possibly represents an effective strategy for EGFR-TKI resistant lung cancer.

3. MDSC can be transformed into an M2 TAM by activation of the RELB gene (subunit of NF-kB), promoting the proliferation and metastasis of tumors and resulting in EGFR-TKI resistance

4. Intratumoral neutrophil density was lower in EGFR-mutant NSCLC in comparison with non-EGFR mutant NSCLC.

5. The abundance of TIBs is significantly higher in NSCLC with the EGFR mutation. Bregs can weaken the response of T cells and NK cells and facilitate the immune-suppressive activity of Tregs by up-regulation of immunoregulatory ligands through secretion of immunosuppressive factors such as IL-10 and TGF-β.

6. TITs in EGFR-mutant NSCLC was lower than in non-EGFR mutated-NSCLC. The number of CD8+ CD39+ T cells in EGFR mutant-NSCLC patients was less than that in wild-type EGFR NSCLC patients. CD39+ T cells were associated with a positive effect of ICIs. The number of CD8+ tumor-infiltrating T cells in EGFR-mutant NSCLC was less than that in wild-type EGFR NSCLC. The proportion of PD-L1+ or PD-1+ in the CD8+ T cell population was also less than non-EGFR mutant NSCLC. TIM3+ T cells in EGFR-mutant NSCLC tumors is fewer than in wild-type EGFR tumors, while T cells that are TIM3 positive can be CD8+ T cells, CD4+ T cells, or NKT cells. PD-L1+ T cells are positively correlated with the poor response of EGFR-TKIs.

7. The relationship between cytokines and EGFR mutation with EGFR-TKI resistance has been investigated, involving complex and diverse signaling pathways, classified generally as tumor EMT.

8. The activation of EGFR in tumor cells increases the activity of MMP-9, which

promotes the destruction of the ECM barrier and the invasiveness of tumor cells. Composition of the ECM is associated with EGFR-TKI resistance. CAFs are therefore associated with EGFR-TKI resistance.

9. The formation of a tumor vascular system is associated with EGFR-TKI resistance.

In addition, future research on reversal of EGFR-TKI resistance is shown in the manuscript. The documentation in the manuscript (summarized in Nos. 1-9, above) was adequate. Thus, I found only one mistake in line 147 (Osimertinib -> osimertinib).

Reply: We appreciate the reviewer's valuable comment. We are sorry for the typo and the writing has been polished.

Changes in the text: We have made the corresponding revision in the revised manuscript (Line 155).

Reviewer B

Comment 1: The authors emphasized the impact of the TIME on resistance to EGFR-TKI. The impact of the TIME on ICIs efficacy in EGFR-mutated NSCLC irrespective intervention of EGFR-TKIs, should be further discussed.

Reply 1: We appreciated the reviewer's valuable and constructive suggestions. There are few studies discussed about the impact of the TIME on ICIs efficacy in EGFR-mutated NSCLC leading to limited information on this topic; however, we have summarised the existed evidence and made some discussions in the "T lymphocytes" section in the revised manuscript.

Changes in the text: According to your suggestion, we have added some content discussing the impact of the TIME on ICIs efficacy in EGFR-mutated NSCLC in the "T lymphocytes" section in the revised manuscript. Please see line 289-306.

Comment 2: In line 484, they should present the literature showing a poor response to ICIs in EGFR-mutated NSCLC.

Reply 2: Thanks for the reviewer's valuable suggestion. According to your comment, we have added the related literature demonstrating the poor response to ICIs in EGFR-mutated NSCLC.

Changes in the text: We have added the related literature demonstrating the poor response to ICIs in EGFR-mutated NSCLC in our revised manuscript (Line 289-293).

Comment 3: The results of the combination of EGFR-TKI and VGFR2 inhibitor have been reported to be useful (Nakagawa K, Garon EB, et al. Lancet Oncol. 2019;20(12):1655-1669). In the section of tumor vasculature, the results of RELAY trial should be mentioned.

Reply 3: We appreciated the reviewer's valuable and constructive suggestion. We have added a description of this clinical trial in the revised manuscript.

Changes in the text: We have added the results of RELAY trial in the revised manuscript (Line 493-496).

Comment 4: Figure legend for Figure3: "MM9" is wrong word.

Reply 4: We appreciate the reviewer's valuable comment. We are sorry for the typo.

Changes in the text: We have carefully checked the spellings throughout our revised manuscript (Line 1136).

Comment 5: Figure legend for Figure3 line8: the authors have to revise "c-met" to "HGF".

Reply 5: Thanks for the reviewer's valuable suggestion. We are sorry for our negligence of this mistake.

Changes in the text: We have carefully checked the spellings throughout our revised manuscript (Line 1140).